MYCOPLASMA PNEUMONIAE AND *BORDETELLA PERTUSSIS* IN PATIENTS WITH PERSISTENT COUGH IN PRIMARY CARE

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I dedicate this thesis to my mother and father.

Thank you for being such wonderful parents and for instilling in me the confidence and tenacity to deal with whatever challenges I may face in life.

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ABSTRACT

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Background

Persistent cough following an acute respiratory tract infection is a challenging and frequently encountered problem in primary care. *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Bordetella pertussis* (pertussis) particularly predispose patients to persistent cough. Whilst the incidence of *M. pneumoniae* is highest in children, pertussis may also occur in adults.

Method

Four studies were conducted for this thesis. First, a systematic review to assess the diagnostic accuracy of symptoms and signs in the clinical recognition of *M. pneumoniae*. Second, a retrospective analysis of a cohort of children with persistent cough to assess the prognostic value of diagnosing *M. pneumoniae*. Third, a prospective cohort study to estimate the prevalence of *M. pneumoniae* and pertussis in children with persistent cough following recent changes in vaccination policy. Fourth, a double-blind randomised placebo-controlled trial to determine the effectiveness of montelukast in the treatment of persistent cough and pertussis-induced cough in adults.

Results

M. pneumoniae and pertussis can each be found in one-sixth of children who present in primary care with persistent cough. Although coverage with the preschool pertussis booster vaccine is high, its efficacy wanes rapidly, with the likelihood of pertussis increasing by 30% per year after vaccination. Montelukast is not an effective treatment for persistent cough, but may be an effective treatment for pertussis-induced cough.

Median duration of cough in children with *M. pneumoniae* is only one-third of that in children with pertussis (39 days versus 118 days). However, the diagnostic accuracy of symptoms and signs in the clinical recognition of *M. pneumoniae* is limited. Since *M. pneumoniae* occurs in cyclical epidemics, clinicians should consider current prevalence of *M. pneumoniae* when making a clinical diagnosis.

Conclusions

Diagnosing *M. pneumoniae* and pertussis can help clinicians give patients an explanation for their cough and inform them about its likely prognosis. At the moment, clinicians should adopt a conservative approach to managing postinfectious persistent cough. A further trial is needed to assess the efficacy of montelukast for the treatment of pertussis-induced cough.

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1 Introduction

The overall aim of this thesis is to provide primary care clinicians with an evidence-based approach to managing patients who present with a postinfectious persistent cough, particularly in relation to *Mycoplasma pneumoniae* (Mp) and *Bordetella pertussis* (also known as pertussis or whooping cough).

I developed an interest in this area as a GP registrar, when I found myself having frequent consultations with children and adults complaining of persistent cough which had been triggered by a cold or chest infection. These patients had almost invariably tried numerous over-the-counter and prescribed medications, none of which had helped their cough. Many had also undergone investigations, such as chest X-rays and spirometry, whose results had all been normal. While this was reassuring on one hand, it was highly unsatisfactory on the other, as the cough would continue to persist and interfere with patients' work, sleep, conversations and exercise, causing them and their families both frustration and concern.

Since there were no proven effective treatments for postinfectious cough which I could suggest, all I could do was reassure these patients or offer to refer them for a second opinion. Unsurprisingly, they were often unhappy with these limited options and felt no closer to a solution for their persistent cough. I therefore set about thinking how I could use my DPhil as an opportunity to improve the way we manage these patients.

This thesis will focus on Mp and pertussis in patients with persistent cough in primary care because these infections particularly predispose patients to persistent cough(1-4). To

develop my specific hypotheses, I have conducted a literature review of our current knowledge in this area (chapter 2).

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2 Background and literature review

2.1 Burden of persistent cough

Persistent cough is a frequently encountered problem in primary care, which results in significant healthcare resource utilisation and socioeconomic burden. A questionnaire survey of UK adults with a persistent cough found that 91% had consulted a general practitioner (GP), 85% had been prescribed medication and 61% had seen at least one hospital specialist for their cough(1). Persistent cough accounts for approximately 20% of referrals to respiratory outpatient clinics(2) and over £500 million is spent on over-the-counter medicines for coughs and colds in the UK each year(3).

However, persistent cough remains a challenging problem to manage in primary care. Coughs may be classified as persistent if they continue for three weeks or longer. 'Acute persistent' or 'subacute' coughs are defined as coughs of three to eight weeks' duration(4), whilst 'chronic' coughs are defined as coughs of more than eight weeks' duration(4-6). Most coughs are acute coughs, which tend to settle within three weeks and are usually caused by viral upper respiratory tract infections (RTIs). However, the differential diagnosis of persistent cough is wider and likely to be influenced by baseline population characteristics and clinical practice.

So far, studies evaluating the causes of chronic cough have focused on patients seen in hospital clinics rather than in primary care(7-10). Adults with chronic cough are most commonly diagnosed with asthma, gastro-oesophageal reflux disease or post-nasal drip(7, 8), while the most common diagnoses in children are lower airway malacia disorders(9)

and protracted bacterial bronchitis(10). However, a diagnosis may not be reached in around 40% of cases; these patients commonly report that their cough started with an acute RTI(8).

Postinfectious cough is likely to account for a substantial proportion of persistent coughs encountered in primary care. A study of 184 non-smoking adults with acute persistent cough diagnosed postinfectious cough in 89 patients (48%). Postinfectious cough was diagnosed when the cough was initiated by an acute RTI and did not meet any other disease criteria(11). Almost 40% of non-smoking non-asthmatic adults have a moderately severe persistent cough a median of three months after an acute respiratory illness(12) and one-tenth of otherwise healthy children who develop an acute RTI are still coughing three weeks later(13). A greater understanding of the postinfectious cough is therefore needed to help improve clinical management of these patients.

2.2 Postinfectious cough

Postinfectious cough is thought to be mediated by persistent airway inflammation and bronchial hyperresponsiveness triggered by acute RTIs(14). Based on the findings of animal studies, unmyelinated vagal afferent nerves known as C-fibres are mainly responsible for initiating cough(15).

The Transient Receptor Potential (TRP) class of ion channels plays an important role in regulating the activation of sensory afferents involved in the cough reflex. TRPA1 (TRP Ankyrin 1) and TRPV1 (TRP Vanilloid 1) ‘cough receptors’ are expressed predominantly in small-diameter nociceptive neurons and are activated by a wide range of endogenous inflammatory mediators and exogenous airway irritants(16). Stimulation of TRPV1 (TRP

Vanilloid 1) is three times more effective than stimulation of TRPA1 channels in evoking sustained activation of tracheal C-fibres in guinea pigs(17).

Bronchial hyperresponsiveness and cough receptor hypersensitivity have been demonstrated in children with recurrent persistent dry cough(18). However, the mechanism of postinfectious cough is likely to be distinct from that of asthma, since, unlike children with untreated asthma, children with postinfectious cough do not have airway eosinophilia(19).

Bordetella pertussis (pertussis) has been reported in 37% of schoolchildren presenting in UK primary care(20) and 20% of adolescents and adults presenting in Canadian health centres(21) with persistent cough. However, *Mycoplasma pneumoniae* (Mp) also predisposes patients, especially children, to persistent cough. Mp was the second most common bacterial pathogen found after pertussis in a prospective cohort of children with persistent cough(22) and a nested cohort of children with persistent cough recruited from participants in a pertussis vaccine trial(23). Mp should therefore also be considered as a potential diagnosis for RTIs and persistent cough, particularly in children.

2.3 *Mycoplasma pneumoniae*

2.3.1 Epidemiology

The highest incidence of Mp is found in school aged children, especially between the ages of 5 and 9 years(24). Mp occurs both endemically and in cyclical epidemics at approximately four-yearly intervals(25). Estimates of Mp prevalence are therefore extremely variable, ranging from 1% during endemic periods(26) to 50% during

outbreaks within closed institutional settings(27). However, the mechanism by which Mp epidemics occur is still uncertain.

Mp isolates can be classified into two main genomic groups (subtypes 1 and 2) based on sequence variation within the P1 adhesin-encoding gene(28). Further sequencing can identify different strains within these subtypes. Predominance of certain strains has been shown to coincide with Mp subtype changes, suggesting a possible mechanism for Mp epidemics(29). However, a recent study in England and Wales found no apparent dominating clonal types and demonstrated a high diversity of Mp strains during a period of increased Mp activity(30). A recent Mp epidemic in Israel and endemic spread of Mp in France were also polyclonal phenomena(31).

2.3.2 Pathogenesis

Mp is an exclusively human pathogen, which resides in close association with respiratory tract epithelial cells. Since Mp lacks a cell wall and has only a small genome, it has limited biosynthetic capabilities and therefore depends on exogenous supplies of amino acids and precursors for RNA and DNA synthesis(32).

Mp attaches to epithelial cells using a specialised attachment organelle. The P1 adhesin protein, which is primarily concentrated at the tip of this organelle, is the main component responsible for Mp-epithelial cell interactions. The close association between Mp and epithelial cells protects it from being removed by the host's mucociliary clearance mechanism and allows it to produce a variety of local cytotoxic effects(33).

The Community Acquired Respiratory Distress Syndrome (CARDS) toxin plays an important role in the pathogenesis of Mp. The CARDS toxin produces ciliostasis, cytoplasmic and nuclear vacuolisation and respiratory epithelial cell fragmentation and sloughing(34) similar to that observed in Mp-infected tracheal organ cultures(35). In Mp-infected mice, CARDS toxin concentration is directly linked to Mp replication and persistence, pro-inflammatory cytokine release and degree of pulmonary inflammation(36, 37). Persistence of CARDS toxin-producing Mp has also been found in adults with refractory asthma(38).

The pathological processes involved in producing Mp-associated extrapulmonary complications are unclear but may involve locally induced cytokines, autoantibody and/or immune complex production and vascular occlusion (vasculitis or thrombosis)(39). Extrapulmonary complications include skin, joint and neurological manifestations.

2.3.3 Clinical manifestations

Mp infections mostly cause mild, self-limiting upper respiratory tract infections, whose onset is gradual over several days(40). Other clinical manifestations include acute pharyngitis,(41) acute wheezing episodes,(42) persistent cough(22, 23) and community-acquired pneumonia(43-45). The clinical severity of Mp infection is associated with higher bacterial load but not with Mp genotype(46).

Cough and fever are the most commonly reported clinical features associated with Mp(40, 47, 48). A five year surveillance study conducted within a large medical co-operative in the United States found that patients with Mp had higher rates of headache, fever, sore throat, skin rashes and ear complications, but lower rates of coryza and leukocytosis than

patients with pneumonia caused by other pathogens(49). However, the authors noted potential sampling bias in that more throat swab and blood samples were obtained from patients who had provisionally been diagnosed with a viral infection rather than bacterial pneumonia.

Previous studies have proposed that Mp may be involved in the pathogenesis of asthma(50) and have reported a higher proportion of persistent(51) and recurrent(52, 53) asthma symptoms in children with acute Mp or *Chlamydia pneumoniae* infections. However, a retrospective clinical cohort study found that Mp infection in children younger than 5 years of age was not associated with long-term effects on airway resistance or bronchial hyperresponsiveness two years later(54).

Extrapulmonary complications may occur in some patients with Mp. Observational studies report Mp in 33% of children with erythema multiforme(55), 32% of children with acute urticaria(56) and 8% of children with erythema nodosum(57). However, the most common rash associated with Mp is a self-limiting maculopapular eruption, which may be localised or confluent(58). Mp is also associated with reactive arthritis, which may manifest as monoarthritis or asymmetric oligoarthritis. Mean recovery time is 4.5 weeks (range 1 to 28 weeks) although progression to chronic juvenile spondyloarthropathy has been reported(59). Neurological complications include encephalitis(60), stroke(61-63), transverse myelitis(64) and Gullain-Barré syndrome(65).

2.3.4 Laboratory diagnosis

At the moment, a diagnosis of Mp can only be confirmed retrospectively using laboratory methods. However, there is no single 'gold standard' for laboratory diagnosis of Mp.

Culture is the most specific method, but can take several weeks, requires special media and expertise, has poor sensitivity and is prone to contaminants and inhibitors(66). Serology is the most widely available method but paired acute and convalescent serum samples taken two to four weeks apart may be required. Furthermore, there is poor agreement between different serological testing kits(67-70).

Polymerase Chain Reaction (PCR) techniques are more rapid and sensitive than serology(71). The reliability of PCR may be enhanced by using it in combination with serology(72) or by using multiple target gene primers(73) including the P1 adhesin gene(74) and the CARDS toxin gene (Mp 181)(73). However, PCR is not currently available as part of routine clinical care in most primary care settings.

2.4 *Bordetella pertussis*

2.4.1 Epidemiology

Pertussis is one of the commonest vaccine preventable diseases, causing nearly 300,000 deaths a year in children worldwide(75). Between 1998 and 2009, pertussis incidence rates in England and Wales were highest in infants younger than 3 months of age and peaked every 3 to 4 years. Household contacts were cited as the source of infection in 95% of infants hospitalised with pertussis(76).

The incidence of pertussis among adolescents and adults is also increasing. In England and Wales, laboratory-confirmed pertussis cases increased by 53% per year and notifications by 18% per year in people aged 15 years and over between 2001 and 2009(76). Pertussis in adolescents and adults is a source of considerable socioeconomic

burden. A two-year study based in the United States found that 83% of adolescents missed school for a mean duration of 5.5 days and 61% of adults missed work for a mean duration of 9.8 days due to pertussis(77).

An accelerated schedule of primary pertussis vaccinations at 2, 3 and 4 months was introduced in the UK in 1990 and primary vaccine coverage has been over 90% since 1992(76). However, the pertussis organism is able to persist in the face of high vaccine coverage by means of virulence-associated gene mutations(78). Immunity against pertussis also wanes with time following pertussis vaccination or infection. Immunity following pertussis vaccination lasts for only 4 to 12 years and immunity post infection for 7 to 20 years(79). This has important implications for pertussis transmission and for the development of serious pertussis-related illness in infants.

To reduce the risk of pertussis transmission between school aged children and infants, a preschool pertussis booster vaccination (PSB) was introduced in the UK in October 2001. The PSB can be administered as a three-component (Infanrix-IPV or Infanrix-IPV-Hib) or a five-component (Repevax) acellular pertussis vaccine in combination with diphtheria, tetanus and inactivated polio vaccines. The five- component vaccine contains pertussis toxoid (PT), filamentous haemagglutinin (FHA), fimbrial agglutinogens (FIM) 2 and 3, and pertactin (PRN). The three-component vaccine contains PT, FHA and PRN(80).

In October 2004, the five-component acellular pertussis vaccine replaced the whole cell pertussis vaccine in the primary vaccination schedule because, although both types of vaccine were equally efficacious, the acellular vaccine was associated with fewer adverse events(81). Pediacel is the recommended vaccine combination product because it

contains a high-dose diphtheria vaccine, whereas Repevax contains a low-dose diphtheria vaccine(80). Between 2000 and 2001, around 50% of infants received Infanrix-IPV-Hib, which contains a three-component acellular pertussis vaccine, because of supply problems with whole cell pertussis vaccine. However, Infanrix-IPV-Hib was subsequently withdrawn from the primary schedule because the acellular vaccine component was found to induce poor immunity to *Haemophilus influenzae* b (Hib)(82).

2.4.2 Pathogenesis

Pertussis is a gram negative coccobacillus, which infects the respiratory tract. The incubation period is 7 to 10 days, following which the illness occurs in three phases. First, a catarrhal prodrome, which may last up to three weeks and mimic a viral RTI. Second, a paroxysmal phase, during which individuals may experience severe bouts (paroxysms) of coughing, whooping and post-tussive vomiting. Third, a convalescent phase during which individuals usually just have a persistent cough. The infectious period lasts from the onset of the catarrhal phase until 21 days after the onset of the paroxysmal phase(75).

Transmission of pertussis is primarily through inhalation. The bacteria adhere to ciliated airway epithelium and produce toxins (including adenylate cyclase toxin, lipopolysaccharide and pertussis toxin), which enhance bacterial multiplication and promote epithelial damage and mucus hypersecretion(83). Bradykinin may also contribute to pertussis-induced cough. Cough initiated by bradykinin is paroxysmal in nature, mimicking the cough associated with pertussis. Lipopolysaccharide and gram negative bacterial infections are both known to initiate bradykinin formation(84).

2.4.3 Clinical manifestations

The World Health Organisation (WHO) defines clinical pertussis as a cough lasting at least two weeks together with at least one of: paroxysms of cough, inspiratory whooping and post-tussive vomiting without any other apparent cause(85). However, only 60% of patients with laboratory-confirmed pertussis and a cough of between 1 and 2 weeks' duration are reported to have at least one of the other features mentioned in the WHO clinical case definition(86).

Duration of cough in children with pertussis (median 112 days, range 38 to 191 days)(20) is longer than that in adults (median 42 days, range 27 to 66 days)(87). Pertussis is also associated with greater severity of cough than non pertussis-related cough. Children with pertussis continue to have more than 5 episodes of cough per day for significantly longer than children without pertussis(20). Adults with pertussis experience a longer duration of violent coughing than adults without pertussis (median 43 days versus 31 days, p=0.0008)(21). Full recovery from the illness and return to previous exercise tolerance may take even longer than the time taken for the cough to resolve(88).

Infants with pertussis are more likely to present with apnoea than infants without pertussis(89). Infants with pertussis are also more likely to have apnoeic episodes than older children and adults with pertussis (Odds Ratio 6.8, 95% confidence interval 1.4 to 64.2)(86). Cyanosis and facial redness during coughing are more common in infants aged less than 6 months than in older children aged 7 to 18 years(90). More importantly, pertussis infection in infants may lead to serious complications including pneumonia, failure to thrive from post-tussive vomiting, seizures, encephalopathy, cerebral hypoxia, secondary bacterial infection, pulmonary hypertension, sub-conjunctival haemorrhage and

rectal prolapse(75).

2.4.4 Laboratory diagnosis

A laboratory-confirmed diagnosis of pertussis can be helpful in terms of giving patients an explanation for their cough and contributing data for public health disease surveillance. Culture of nasopharyngeal aspirate is the gold standard laboratory method for diagnosing pertussis. However, it can take up to 7 days to obtain a result and sensitivity is poor (15% to 45% if a sample is obtained within 21 days of the onset of cough, 0% if obtained 3 weeks or longer after the onset of cough)(75). Bacterial load may also decline below the diagnostic threshold of PCR in patients with a cough of more than three weeks' duration(91). The sensitivity of PCR is 73% to 100% depending on the gene target used(75). However, its specificity is difficult to evaluate given the poor sensitivity of culture(92).

Serology is the most reliable method of diagnosing pertussis in patients who present more than three weeks after the onset of cough. Measurement in ELISA (enzyme-linked immunosorbent assay) of IgG antibodies to pertussis toxin (IgG-PT) is the method of choice. A large population-based study found that an IgG-PT titre of >=100 units (U)/ml on a single serum sample was diagnostic of pertussis; only 0.8% of the general population had IgG-PT levels >=100 U/ml(93). A more recent study found that a >=3-fold increase in IgG-PT to a level of >=20 international units (IU)/ml or a >=2-fold increase to a level of >100 IU/ml were diagnostic of pertussis(94).

An IgG-capture ELISA capable of detecting anti-PT IgG in oral fluid has also been developed. A previous study, which compared 187 matched serum and oral fluid samples

from children with persistent cough reported that an oral fluid IgG-PT titre of >=70 arbitrary units had a sensitivity of 80% and a specificity of 97% compared with serum ELISA(95). This assay has since been further developed to improve its performance, giving a sensitivity of 93% and a specificity of 94% compared to serum ELISA(96).

2.5 Clinical management of postinfectious cough

The management of postinfectious cough in primary care is challenging because diagnosing the causative pathogen on initial presentation can be difficult and there are currently no proven effective treatments(2). In particular, there is insufficient high quality trial evidence relating to the effectiveness of treatments for postinfectious cough.

A multicentre double-blind trial conducted in non-smoking adults with postinfectious cough randomly allocated patients to a five-day treatment course of SCH486757, codeine or placebo(97). SCH486757 is a nociceptin opioid 1 (NOP1) receptor agonist, which has been demonstrated to have significant antitussive activity in guinea pigs(98, 99). There were no significant changes in average cough severity scores or objective cough counts between either SCH486757 or codeine versus placebo. However, the trial was underpowered due to difficulties with recruitment.

A double-blind crossover trial of inhaled ipratropium bromide for post viral cough in non-smoking adults found that day-time and night-time cough severity were significantly lower after a three-week course of inhaled ipratropium bromide versus placebo(100). However, the clinical significance of these findings is uncertain, since the trial sample size was small (n=14) and cough severity was measured using subjective patient-reported symptom scores, ranging from 0 (no symptoms) to 3 (severe symptoms). Furthermore,

differences in cough scores after treatment with inhaled ipratropium bromide versus placebo were only modest (day-time cough 1.29 (ipratropium bromide) versus 1.66 (placebo); night-time cough 0.82 (ipratropium bromide) versus 1.24 (placebo)).

A larger randomised placebo-controlled trial of inhaled fluticasone for adults with persistent cough (n=133), mostly subacute cough (n=89), used the same patient-reported cough severity scoring system(101). This trial only demonstrated a modest reduction in cough severity after two weeks of fluticasone treatment in non-smokers (n=84) (change in daily cough score 0.9 points, 95% confidence interval 0.4 to 1.3).

Another trial found that mean changes in cough symptom scores after two and four weeks were not significantly different between non asthmatic, non smoking patients with postinfectious persistent cough treated with inhaled budesonide versus placebo(102). Cough symptom scores encompassed patient-reported ratings for frequency of cough, frequency of coughing bouts, symptoms associated with cough, night-time cough and frequency and number of medications taken to relieve cough.

Macrolide antibiotics are the recommended treatment for Mp infections; beta-lactams are not recommended because the organism has no cell wall(103). Macrolide antibiotics are also recommended in clinically suspected and laboratory-confirmed pertussis cases if detected within 21 days of the onset of cough(104). In these patients, antibiotics shorten the duration of the infectious period, but not the duration of cough(105). There are currently no proven efficacious treatments for pertussis-induced cough(106).

A recent systematic review examined the efficacy of various treatments in reducing the severity of paroxysmal cough in patients with pertussis(106). The review found two placebo controlled trials of salbutamol(107, 108), one of diphenhydramine(109) and one of pertussis immunoglobulin(110). The number of paroxysms of cough per 24 hours(107-109) and the mean number of paroxysmal coughing episodes per hour(110) were used as outcome measures. Based on published data, none of these treatments were significantly more efficacious than placebo.

2.6 Summary and thesis planning

This literature review highlights the need for further research to help primary care clinicians diagnose Mp and pertussis more accurately. This chapter also demonstrates a substantial gap in the evidence base relating to treatments for postinfectious and pertussis-induced cough. For this thesis, I identified several opportunities to conduct further research in these areas at the Department of Primary Care Health Sciences in Oxford.

Firstly, I identified colleagues in the department with strong methodological expertise in conducting diagnostic systematic reviews. I also found out that the Cochrane Diagnostic Test Accuracy Group based at the University of Birmingham offered a series of workshops and methodological support for authors of Cochrane-registered diagnostic test accuracy reviews. I therefore decided to perform a systematic review to assess the diagnostic accuracy of symptoms and signs in the clinical recognition of Mp in children and adolescents and registered the title of this review with the Cochrane Acute Respiratory Infections Group.

Secondly, I identified the need for a retrospective analysis of cough duration data and microbiological samples from a prospectively recruited cohort of children with persistent cough who presented in primary care between October 2001 and March 2005. Only data on pertussis prevalence and duration of cough had already been published(20). However, nasopharyngeal aspirates had also been obtained from these children, which had not previously been analysed. The study investigators kindly gave me access to the original dataset. In addition, colleagues at the London Health Protection Agency kindly agreed to analyse the blood and nasopharyngeal aspirates from this cohort to detect Mp and respiratory viruses. I therefore decided to use these data to assess the prognostic value of detecting Mp and respiratory viruses in children with persistent cough.

Thirdly, one of my supervisors (Dr Harnden) discussed with me a trial which he had been planning to determine the efficacy of montelukast for the treatment of persistent cough due to pertussis. I took the opportunity to develop this into a trial to determine the effectiveness of montelukast for postinfectious persistent cough, which would also assess the feasibility and practicalities of conducting a trial to determine the efficacy of montelukast for pertussis-induced cough. I felt that the results of a trial in patients with postinfectious cough would be of more direct clinical relevance than a trial in patients with pertussis-induced cough because many primary care clinicians do not have routine access to laboratory investigations for pertussis. However, postinfectious cough can be diagnosed based on clinical assessment during the initial consultation.

Although the original intention had been to conduct the trial in children and adults, I was only able to conduct it in adults because there was no suitable placebo available for montelukast in chewable tablet or granule forms, which are recommended for patients

aged 15 years and younger. I therefore decided instead to perform a prospective cohort study in children with persistent cough to estimate the prevalence of Mp and pertussis following recent changes in UK childhood vaccination policy, particularly the introduction of the PSB.

Chapter 3 gives an overview of the overall structure and specific objectives of this thesis.

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3 Overall structure and objectives of thesis

Chapter 4: Clinical symptoms and signs for the diagnosis of *Mycoplasma pneumoniae* in children and adolescents with community-acquired pneumonia: a systematic review.

This chapter assesses the diagnostic accuracy of symptoms and signs in the clinical recognition of *Mycoplasma pneumoniae* in children and adolescents with community-acquired pneumonia based on data from published studies.

Chapter 5: *Mycoplasma pneumoniae* and respiratory virus infections in children with persistent cough: a retrospective analysis.

This chapter assesses the prognostic value of detecting Mp and respiratory viruses in children with persistent cough by retrospectively analysing blood samples, nasopharyngeal aspirates and cough duration data from children who presented in primary care with persistent cough between October 2001 and March 2005.

Chapter 6: Prevalence of *Bordetella pertussis* and *Mycoplasma pneumoniae* in children with persistent cough in primary care: a prospective cohort study.

Following recent changes in UK vaccination policy, this chapter estimates the current prevalence of pertussis and Mp in a cohort of children who presented with persistent cough in primary care between November 2010 and March 2012. This chapter also assesses baseline patient characteristics as potential predictors of these infections.

Chapter 7: Montelukast for the treatment of persistent cough in young people and adults: a double-blind randomised placebo-controlled trial.

This chapter is a double-blind randomised placebo-controlled trial to determine whether montelukast is an effective treatment for persistent cough in young people and adults aged 16 to 49 years. This chapter also assesses the feasibility and practicalities of performing a further trial to determine the efficacy of montelukast in the treatment of persistent cough in young people and adults with pertussis.

Chapter 8: Conclusions.

This chapter summarises the overall findings of this thesis and discusses them in the context of the existing literature. This chapter also discusses the implications of these findings for clinical practice and future research.

4 Clinical symptoms and signs for the diagnosis of *Mycoplasma pneumoniae* in children and adolescents with community-acquired pneumonia: a systematic review

4.1 Introduction

4.1.1 Background and rationale

Mycoplasma pneumoniae (Mp) is an important cause of respiratory tract infections in children and adolescents. Data from previous studies suggest that Mp is responsible for up to 40% of community-acquired pneumonia (CAP) in children over 5 years of age(1-3).

Accurate diagnosis of Mp is important to facilitate appropriate antibiotic prescribing. Although amoxicillin is often the first-line empirical antibiotic used in children with CAP, macrolide antibiotics are the recommended treatment for Mp because the organism has no cell wall(4).

Diagnostic uncertainty can lead to inappropriate antibiotic prescribing, which may worsen clinical prognosis and increase antibiotic resistance within both communities(5) and individuals(6). However, as discussed in chapter 2, a diagnosis of Mp can only be confirmed retrospectively, usually by serology.

This review aimed to determine whether clinical symptoms and signs can be reliably used by clinicians to help them decide which children with clinically suspected CAP are most likely to benefit from empirical macrolide treatment at the time of initial presentation, when the results of laboratory tests for Mp are not available.

4.1.2 Objectives

1. To assess the diagnostic accuracy of symptoms and signs in the clinical recognition of Mp in children and adolescents with CAP.
2. To assess the influence of potential sources of heterogeneity on the diagnostic accuracy of symptoms and signs in the clinical recognition of Mp in children and adolescents with CAP.

4.2 Methods

4.2.1 Criteria for study inclusion

This review included published peer-reviewed studies (any design), which prospectively and consecutively recruited participants from any healthcare setting. Participants were aged 18 years or younger with no evidence of serious underlying co-morbidity (*e.g.* cystic fibrosis, bronchiectasis, neoplasia) or immunocompromise (HIV-positive or on immunosuppressant medication) and had been diagnosed with CAP based on clinical +/- radiological criteria.

Included studies reported clinical symptoms and signs in sufficient detail to construct 2x2 tables. This review studied the following clinical symptoms and signs: cough, wheeze, coryza, crepitations, fever, rhonchi, shortness of breath, headache, chest pain, diarrhoea and myalgia. Included studies also confirmed Mp infection using serology with or without additional laboratory tests such as culture and polymerase chain reaction (PCR). In this review, the reference standard was a positive Mp serology result (*i.e.* a high antibody titre on a single serum sample or a significant rise in antibody titre between

paired acute and convalescent sera) with or without additional confirmation using other laboratory methods.

Case series, systematic reviews and narrative reviews were excluded. Studies with unsuitable comparison groups (*i.e.* non consecutively recruited Mp-negative controls or participants with a different laboratory-confirmed microbial diagnosis) were also excluded because assessments of the diagnostic value of symptoms and signs are likely to be distorted in these types of populations.

4.2.2 Search methods

I worked with two information specialists to formulate electronic search strategies for MEDLINE and EMBASE. Appendix 1 contains details of these search strategies. No language or publication restrictions were applied to the search. An information specialist performed searches of MEDLINE (January 1950 to 26th June 2012) and EMBASE (January 1980 to 26th June 2012).

I identified additional studies by handsearching the reference lists of included studies and relevant systematic reviews and by snowballing. I also searched the following databases to identify systematic reviews whose reference lists might provide additional references: the Medion database (http://mediondatabase.nl) (25th June 2012), The Cochrane Library's Database of Reviews of Effects 2012, Issue 6 (25th June 2012) and the Cochrane Register of Diagnostic Test Accuracy studies (2nd July 2012). I asked experts in the field to review the list of included studies for any obvious omissions.

4.2.3 Selection of studies

I scanned the titles of studies identified by the search to exclude any obviously irrelevant articles. I and another review author then independently scanned the titles and abstracts of the remaining studies and reviewed full-text versions of potentially relevant articles. We resolved any disagreements by discussion, if necessary with a third author.

4.2.4 Data extraction and management

I and another review author independently extracted data on the following study characteristics: study design, age and sex of participants, study inclusion and exclusion criteria, number of participants recruited, recruitment period, country(ies) where recruitment took place, healthcare setting, criteria for diagnosing CAP, laboratory methods used to diagnose Mp, number of participants diagnosed with Mp and clinical symptoms and signs. We also independently constructed 2x2 tables cross-classifying the absence or presence of Mp with the absence or presence of clinical symptoms and signs.

To assess study quality, I developed a quality assessment tool and coding criteria based on the QUADAS tool(7) (Appendix 2). I and another review author independently assessed the quality of included studies using this tool. We resolved any discrepancies in construction of 2x2 tables or quality assessment by discussion, if necessary with a third author.

4.2.5 Statistical analysis

I performed the statistical analysis of data for this review. Study-specific values for sensitivity, specificity and positive and negative likelihood ratios were calculated with 95% confidence intervals. The influence of Mp prevalence in the study population on the

post-test probability of Mp was also examined based on the absence or presence of different symptoms and signs(8).

For clinical symptoms and signs where data were reported by at least four included studies, pooled sensitivities, specificities, positive and negative likelihood ratios with 95% confidence intervals were calculated by fitting a bivariate normal model for the logit transforms of sensitivity and specificity(9). Estimates from these models were obtained using the command *metandi* (meta-analysis of diagnostic accuracy) in Stata version 11. Summary Receiver Operating Characteristic (ROC) curves with 95% confidence regions for summary points were also plotted using RevMan version 5.1.

I planned to explore the following potential sources of heterogeneity by fitting bivariate models with covariates using multi-level mixed-effects logistic regression (*xtmelogit*) in Stata version 11:

1. Participant age group (preschool (up to 4 years) versus school age (5 to 12 years) versus adolescents (13 to 18 years)).
2. Healthcare setting (community versus hospital).
3. Method of diagnosing CAP (based on clinical criteria only versus based on clinical and radiological criteria).
4. Serological method of diagnosing Mp (high antibody titre on single serum sample versus significant rise in antibody titre between acute and convalescent sera).
5. Use of other laboratory investigations alongside serology to diagnose Mp.

Sensitivity analyses were performed to explore the influence of negative classification of items 1 (representative spectrum) and 2 (acceptable reference standard) of the quality

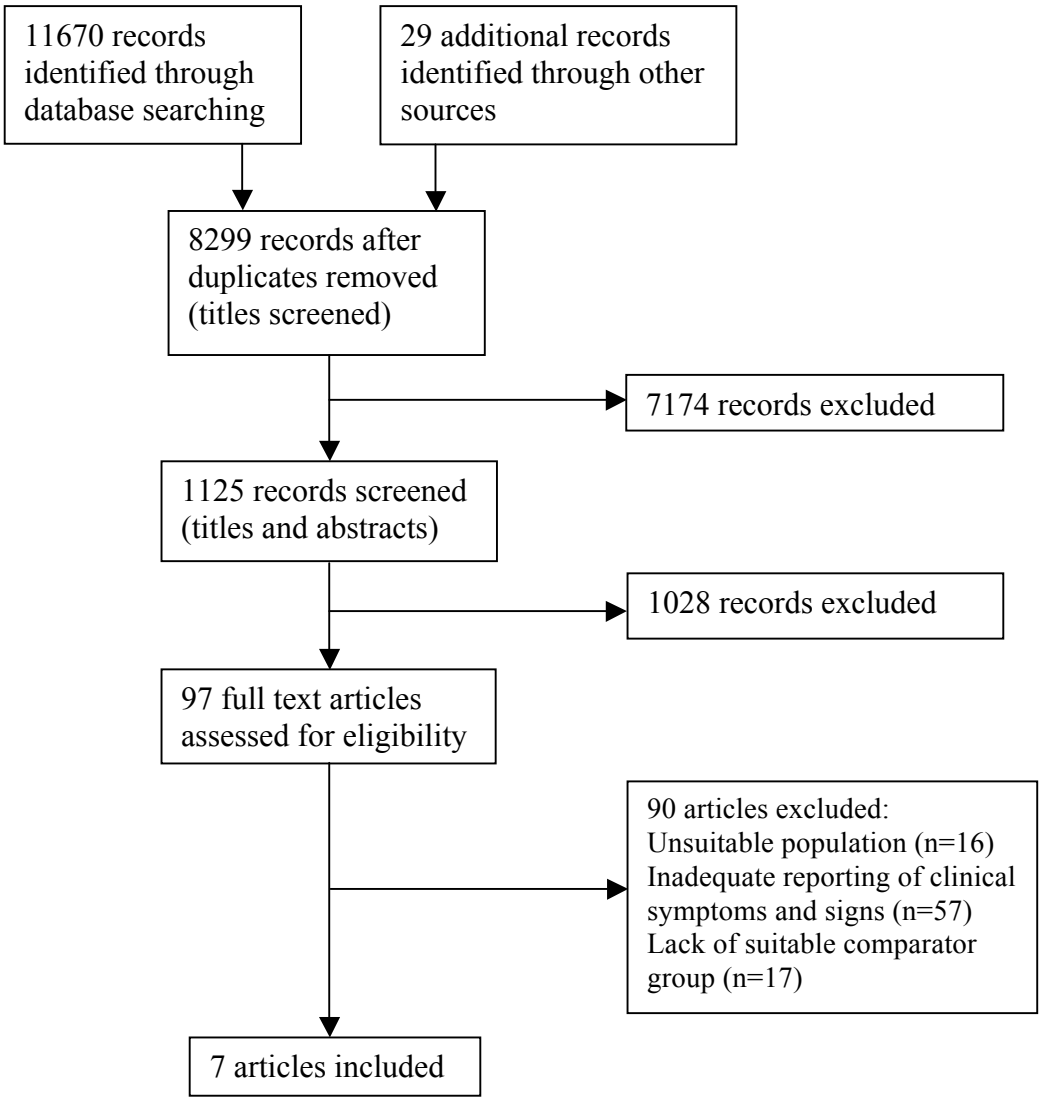
assessment tool (Appendix 2). No formal assessment of reporting bias was undertaken due to current uncertainty about how to assess reporting bias in diagnostic test accuracy reviews(10).

4.3 Results

4.3.1 Results of the search

Figure 1 summarises the numbers of articles identified, screened and selected for this review. The search identified 8299 articles (excluding duplicates) of which 7174 were excluded based on title alone. A further 1028 articles were excluded after reviewing their abstracts. Full text versions of the remaining 97 articles were reviewed. Seven articles were included in this review. Agreement between the other review author and me was very good based on title and abstract screening (percentage agreement = 97.2%; kappa = 0.84) and good based on full-text screening (percentage agreement = 93.8%; kappa = 0.67).

Figure 1: Study flow diagram



4.3.2 Characteristics of excluded studies

Of the 97 full text articles assessed for eligibility, 90 were excluded. The three main reasons for exclusion were: unsuitable population (n=16), inadequate reporting of clinical symptoms and signs (n=57) and lack of suitable comparator group (n=17).

Unsuitable population

Three studies were performed in adult populations(11-13). Two studies were excluded because their populations were considered highly likely to include children with serious

co-morbidity or immunocompromise. In one study(14), mean duration of hospitalisation was 18.8 days and 25% of children developed respiratory failure. In another study(15) 23% of children with Mp and 17% without Mp required mechanical ventilation.

One study did not recruit children with CAP(16). Four studies recruited patients with acute respiratory infections including pneumonia but did not report symptoms and signs in patients with CAP specifically(17-20). One study did not recruit children consecutively; only children with CAP in whom bacterial pathogens, Mp and *Chlamydia pneumoniae* were felt to be the causative organisms after clinical examination were included(21). In two studies, no Mp cases were detected(22, 23). Three studies did not perform laboratory tests for Mp(24-26).

Inadequate reporting of clinical symptoms and signs

Thirty-three articles were excluded because they did not report any data on clinical symptoms or signs(3, 27-58). A further 24 articles were also excluded because they did not report data on clinical symptoms and signs in sufficient detail to construct 2x2 tables(59-82).

Lack of suitable comparator group

Twelve studies were case series with no comparator groups, which only reported data on clinical features in patients with Mp(83-94). Five studies had unsuitable comparison groups consisting of patients with *Chlamydia pneumoniae*(95-98) or other microbial diagnoses(99).

4.3.3 Characteristics of included studies

This review included seven studies, which reported data on clinical symptoms and signs in a total of 1491 children with CAP(100-106). The prevalence of Mp ranged from 10%(100) to 36%(103, 105). All included studies were prospective observational cohort studies conducted in hospital settings. One study was nested within a larger prospective study evaluating the incidence of bacterial and atypical pathogens in hospitalised children with CAP(106).

One study recruited children with severe CAP based on clinical features(100). Another study diagnosed children with CAP based on the presence of respiratory symptoms and respiratory signs or chest radiograph changes(101). All other included studies diagnosed children with CAP based on both clinical and radiographic features(102-106). Two studies only used serology to diagnose Mp(101, 106). Four studies also used PCR of respiratory samples to detect Mp: one study analysed throat swabs(104), two studies analysed nasopharyngeal aspirates (NPAs)(103, 105) and one study analysed NPAs, sputum and throat swabs(102). One study performed Mp antigen detection in NPA in addition to serology(100). This was also the only study which diagnosed Mp based on a single acute serum sample(100). All other included studies sought to obtain paired serum samples from participants.

4.3.4 Methodological quality of included studies

Table 1 summarises the methodological quality of studies included in this review. Only two studies clearly reported that children with serious underlying co-morbidity or immunocompromise were excluded from the study population(105, 106). One study included 51/245 children with asthma (21%) but only nine children (4%) with serious co-

morbidities (seven children had congestive heart failure, one had hepatic disease and one had renal impairment)(102). In three studies it was unclear whether or not a representative spectrum of patients had been recruited(101, 103, 104). These studies did not state whether or not children with co-morbid conditions were excluded or report data on co-morbidities or clinical outcomes. One study only recruited children with severe CAP based on World Health Organisation (WHO) clinical criteria(100); we therefore did not consider this study population to reflect a representative spectrum of children with CAP.

All studies except one(100) utilised acceptable reference standards for diagnosing Mp. In two studies Mp was diagnosed based on a single high antibody titre or a fourfold rise in antibody titre between acute and convalescent serum samples taken two to four weeks apart(101, 106). One study diagnosed Mp based on a fourfold rise in antibody titre between acute and convalescent sera or a positive PCR result together with a persistently high antibody titre(102). Children with a positive PCR result in the absence of serological evidence of Mp infection were considered to be carriers of Mp and were therefore not categorised as having current Mp infection.

In three studies children with positive PCR results were diagnosed with Mp even in the absence of positive serology results(103-105). However, the numbers children who tested positive for Mp on PCR but not on serology were low. In one study PCR was positive in 20 children of whom only three did not have serological evidence of Mp(103). In another study PCR was positive in five children of whom only one did not have serological evidence of Mp(104). Another study reported that 16 children with community-acquired lower respiratory tract infections (acute bronchitis, wheezing or pneumonia) had positive PCR results without serological evidence of acute infection(105). The study did not report

how many of these children were in the pneumonia subgroup, of whom 36% (150/418) were diagnosed with Mp. However, even if all 16 children had been in this subgroup, they would only have accounted for 11% of Mp diagnoses in children with CAP.

One study used antigen detection of Mp in NPA alongside serology as its reference standard(100). However, it was unclear whether or not this reference standard was acceptable, as no children tested positive for Mp using both laboratory methods. Of the 24 children who were diagnosed with Mp, 14 were positive by serology and 10 by antigen detection in NPA.

All included studies avoided partial and differential verification. One study obtained paired serum samples from 245/257 children(102); the 12 children from whom convalescent serum samples could not be obtained were excluded from the study population. Another study obtained paired serum samples from 140/159 children; the 19 children from whom convalescent serum samples could not be obtained were excluded from the study population(106). Another study sought to obtain convalescent serum samples from all 75 children who entered the study but only managed this in 45 children(104). Children from whom convalescent serum samples were not obtained were still included in the study population. Only 2/23 children with Mp were diagnosed on the basis of a fourfold rise in antibody titre alone.

Only one study clearly reported that acute serum samples were obtained within 24 hours of hospital admission, when clinical symptoms and signs were assessed(106). It was not possible to assess whether or not the delay between assessment of clinical symptoms and signs and obtaining samples for Mp detection was acceptable in five studies, as these did not report the time interval between clinical assessment and sample-taking(100-104).

Another study reported that symptoms and signs were recorded at the time of hospital admission whereas laboratory samples were taken at the time of enrolment into the study(105). The mean duration of hospitalisation ranged from 5.68 days in children with neither Mp nor *Chlamydia pneumoniae* infection to 6.63 days in children with both infections. We therefore considered that the delay between clinical assessment and laboratory sample taking was unacceptable in this study.

All included studies avoided incorporation bias, as Mp was diagnosed based on laboratory test results and not on the absence or presence of clinical symptoms or signs. Blinding of the reference standard also took place in all included studies. One study reported that clinical assessment was performed at the time of hospital admission, when the results of convalescent serum samples would not have been available(106). Five other studies also sought convalescent serum samples from children, the results of which would not have been available during the acute illness episode, when clinical symptoms and signs were recorded(101-105). One study only obtained acute laboratory samples but the results of these would not have been available on admission, when clinical symptoms and signs were recorded(100).

No studies explicitly reported that interpretation of laboratory tests was performed blinded to knowledge about clinical symptoms and signs. However, this is unlikely to have occurred in four studies, which specified clear laboratory criteria and antibody titre thresholds for the diagnosis of Mp(101, 102, 105, 106). Blinding to clinical symptoms and signs was unclear in three studies, which did not report diagnostic antibody titre thresholds(100, 103, 104). No studies reported borderline or uninterpretable serology results. The five studies which used additional laboratory methods alongside serology to diagnose Mp all reported data on participants with discrepant results on different

tests(100, 102-105). However, one study did not report the number of discrepant test results within the pneumonia subgroup(105).

Table 1: Methodological quality of included studies

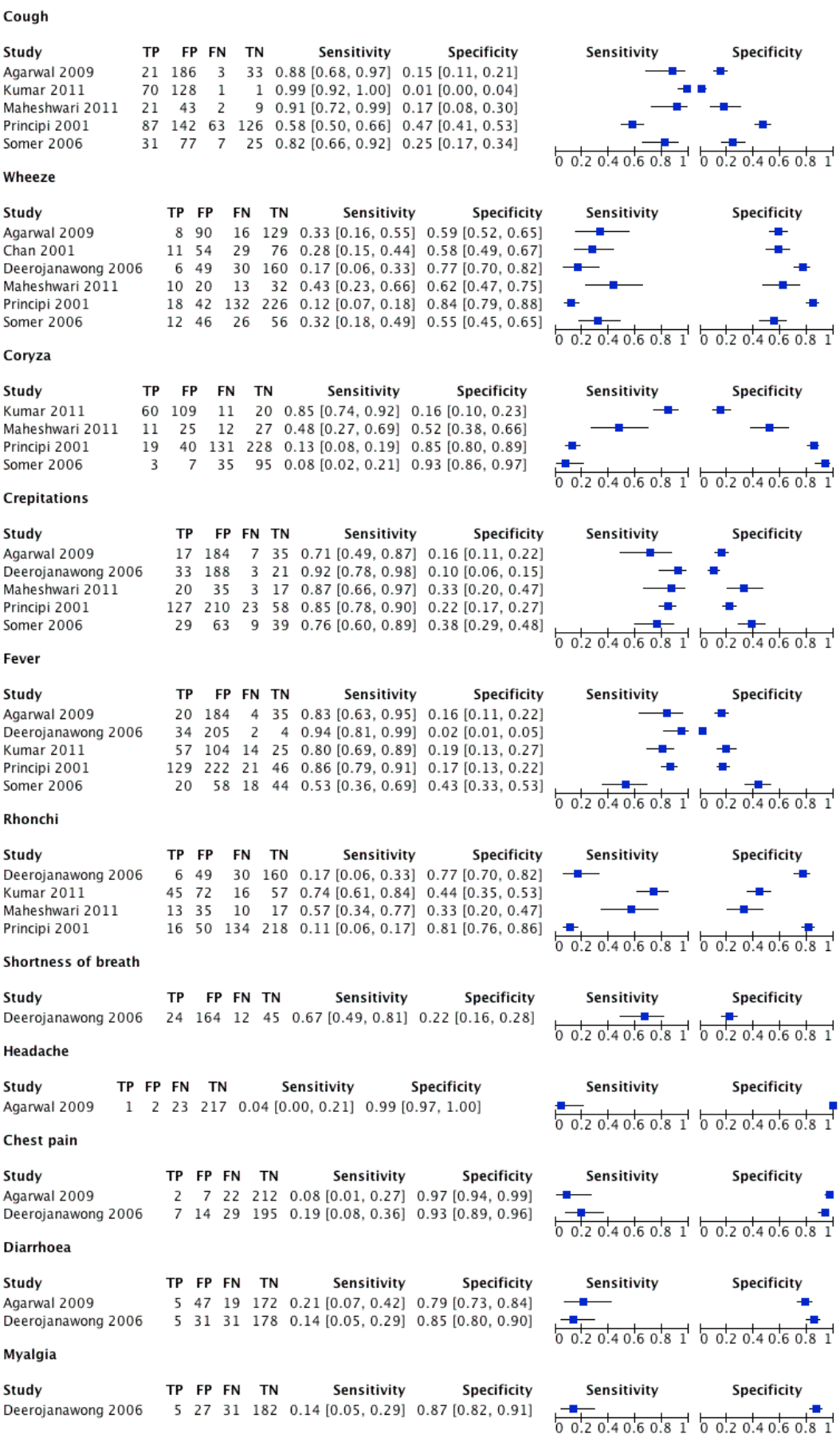
	Quality assessment tool item										
Study	1	2	3	4	5	6	7	8	9	10	11
Agarwal 2009	-	?	?	+	+	+	+	?	?	-	+
Chan 2001	?	+	?	+	+	+	+	+	+	-	+
Deerojanawong 2006	+	+	?	+	+	+	+	+	?	+	+
Kumar 2011	?	+	?	+	+	+	+	?	?	+	+
Maheshwari 2011	?	+	?	+	+	+	+	?	+	+	+
Principi 2001	+	+	-	+	+	+	+	+	+	?	+
Somer 2006	+	+	+	+	+	+	+	+	+	-	+

Quality assessment tool items rated 'Yes' (+), 'No' (-) or 'Unclear' (?).
Summary of items: 1. Representative spectrum of patients; 2. Acceptable reference standard; 3. Acceptable delay between tests; 4. Partial verification avoided; 5. Differential verification avoided; 6. Incorporation avoided; 7. Reference standard results blinded; 8. Index test results blinded; 9. Relevant clinical information available; 10. Uninterpretable results reported; 11. Withdrawals explained.
Appendix 2 outlines details of the quality assessment tool and coding criteria used.

4.3.5 Findings

Figure 2 summarises study-specific values for the sensitivities and specificities of cough, wheeze, coryza, crepitations, fever, rhonchi, shortness of breath, chest pain, diarrhoea and myalgia with 95% confidence intervals. For cough, coryza, fever and rhonchi, sensitivity and specificity varied widely between different studies. In particular, the variation in study-specific specificity values was 20-fold for fever (0.02 to 0.43) and 50-fold for cough (0.01 to 0.47). There was a 10-fold variation in study-specific sensitivity for coryza (0.08 to 0.85).

Figure 2: Study-specific sensitivities and specificities with 95% confidence intervals



TP=True Positive, FP=False Positive, FN=False Negative, TN=True Negative

The pre-test probabilities of Mp in our included study populations were: Agarwal 2009(100): 0.10 (95% confidence interval (CI) 0.06 to 0.14); Chan 2001(101): 0.24 (95% CI 0.17 to 0.31); Deerojanawong 2006(102): 0.15 (95% CI 0.11 to 0.20); Kumar 2011(103): 0.35 (95% CI 0.29 to 0.42); Maheswari 2011(104): 0.31 (95% CI 0.21 to 0.42); Principi 2001(105): 0.36 (95% CI 0.31 to 0.41); Somer 2006(106): 0.27 (95% CI 0.20 to 0.35). Table 2 shows that rhonchi were 32% more likely to be present in children with Mp in Kumar 2011(103), but 33% more likely to be absent in children with Mp in Maheshwari 2011(104). In two studies the presence of chest pain more than doubled the probability of Mp(100, 102). The presence of chest pain increased the probability of Mp from 10% to 22% in one study(100) and from 15% to 33% in the other study(102).

Table 2: Post-test probabilities and likelihood ratios with 95% confidence intervals
CI = confidence interval

Study (first author and year of publication)	Post-test probability - symptom or sign positive (95% CI)	Post-test probability – symptom or sign negative (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
Cough				
Agarwal 2009	0.10 (0.06 to 0.15)	0.08 (0.02 to 0.22)	1.03 (0.88 to 1.21)	0.83 (0.28 to 2.50)
Kumar 2011	0.35 (0.29 to 0.42)	0.50 (0.01 to 0.99)	0.99 (0.96 to 1.03)	1.82 (0.12 to 28.6)
Maheshwari 2011	0.33 (0.22 to 0.46)	0.18 (0.02 to 0.52)	1.10 (0.93 to 1.32)	0.50 (0.12 to 2.15)
Principi 2001	0.38 (0.32 to 0.45)	0.33 (0.27 to 0.41)	1.09 (0.92 to 1.31)	0.89 (0.71 to 1.12)
Somer 2006	0.29 (0.20 to 0.38)	0.22 (0.09 to 0.40)	1.08 (0.90 to 1.30)	0.75 (0.36 to 1.60)
Wheeze				
Agarwal 2009	0.08 (0.04 to 0.15)	0.11 (0.06 to 0.17)	0.81 (0.45 to 1.46)	1.13 (0.84 to 1.53)
Chan 2001	0.17 (0.90 to 0.28)	0.28 (0.16 to 0.55)	0.66 (0.39 to 1.14)	1.24 (0.98 to 1.58)
Deerojanawong 2006	0.11 (0.04 to 0.22)	0.16 (0.11 to 0.22)	0.71 (0.33 to 1.54)	1.09 (0.92 to 1.28)
Maheshwari 2011	0.33 (0.17 to 0.53)	0.29 (0.16 to 0.44)	1.13 (0.63 to 2.02)	0.92 (0.61 to 1.40)
Principi 2001	0.30 (0.19 to 0.43)	0.37 (0.32 to 0.42)	0.77 (0.46 to 1.28)	1.04 (0.97 to 1.13)
Somer 2006	0.21 (0.11 to 0.33)	0.32 (0.22 to 0.43)	0.70 (0.42 to 1.17)	1.25 (0.94 to 1.65)

Table 2 continued

Study (first author and year of publication)	Post-test probability - symptom or sign positive (95% CI)	Post-test probability - symptom or sign negative (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
Coryza				
Kumar 2011	0.36 (0.28 to 0.43)	0.35 (0.19 to 0.55)	1.00 (0.88 to 1.13)	1.00 (0.51 to 1.97)
Maheshwari 2011	0.31 (0.16 to 0.48)	0.31 (0.17 to 0.48)	0.99 (0.60 to 1.66)	1.00 (0.51 to 1.97)
Principi 2001	0.32 (0.21 to 0.46)	0.36 (0.32 to 0.42)	0.85 (0.51 to 1.41)	1.03 (0.95 to 1.11)
Somer 2006	0.30 (0.07 to 0.65)	0.27 (0.20 to 0.35)	1.15 (0.31 to 4.22)	0.99 (0.89 to 1.10)
Crepitations				
Agarwal 2009	0.08 (0.05 to 0.13)	0.17 (0.07 to 0.31)	0.84 (0.65 to 1.10)	1.83 (0.91 to 3.65)
Deerojanawong 2006	0.15 (0.11 to 0.20)	0.13 (0.03 to 0.32)	1.02 (0.91 to 1.14)	0.83 (0.26 to 2.64)
Maheshwari 2011	0.36 (0.24 to 0.50)	0.15 (0.03 to 0.38)	1.29 (1.01 to 1.65)	0.40 (0.13 to 1.23)
Principi 2001	0.38 (0.32 to 0.43)	0.28 (0.19 to 0.40)	1.08 (0.99 to 1.19)	0.71 (0.46 to 1.10)
Somer 2006	0.32 (0.22 to 0.42)	0.19 (0.09 to 0.33)	1.24 (0.98 to 1.56)	0.62 (0.33 to 1.15)
Fever				
Agarwal 2009	0.10 (0.06 to 0.15)	0.10 (0.03 to 0.24)	0.99 (0.82 to 1.20)	1.04 (0.41 to 2.68)
Deerojanawong 2006	0.14 (0.10 to 0.19)	0.33 (0.04 to 0.78)	0.96 (0.89 to 1.05)	2.90 (0.55 to 15.27)
Kumar 2011	0.35 (0.28 to 0.43)	0.36 (0.21 to 0.53)	1.00 (0.86 to 1.15)	1.02 (0.57 to 1.83)
Principi 2001	0.37 (0.32 to 0.42)	0.31 (0.21 to 0.44)	1.04 (0.95 to 1.13)	0.82 (0.51 to 1.31)
Somer 2006	0.26 (0.16 to 0.37)	0.29 (0.18 to 0.42)	0.93 (0.66 to 1.31)	1.10 (0.73 to 1.64)
Rhonchi				
Deerojanawong 2006	0.11 (0.04 to 0.22)	0.16 (0.11 to 0.22)	0.71 (0.33 to 1.54)	1.09 (0.92 to 1.28)
Kumar 2011	0.38 (0.30 to 0.48)	0.22 (0.13 to 0.33)	1.32 (1.07 to 1.64)	0.59 (0.37 to 0.94)
Maheshwari 2011	0.27 (0.15 to 0.42)	0.37 (0.19 to 0.58)	0.84 (0.56 to 1.26)	1.33 (0.72 to 2.44)
Principi 2001	0.24 (0.15 to 0.36)	0.38 (0.33 to 0.43)	0.57 (0.34 to 0.97)	1.10 (1.01 to 1.19)
Shortness of breath				
Deerojanawong 2006	0.13 (0.08 to 0.18)	0.21 (0.11 to 0.34)	0.85 (0.67 to 1.08)	1.55 (0.91 to 2.63)
Headache				
Agarwal 2009	0.04 (0.00 to 0.21)	0.01 (0.00 to 0.03)	4.56 (0.43 to 48.48)	0.97 (0.89 to 1.05)
Chest pain				
Agarwal 2009	0.22 (0.03 to 0.60)	0.09 (0.06 to 0.14)	2.61 (0.57 to 11.85)	0.95 (0.84 to 1.07)
Deerojanawong 2006	0.33 (0.15 to 0.57)	0.13 (0.09 to 0.18)	2.90 (1.26 to 6.69)	0.86 (0.73 to 1.02)
Diarrhoea				
Agarwal 2009	0.10 (0.03 to 0.21)	0.10 (0.06 to 0.15)	0.97 (0.43 to 2.20)	1.01 (0.81 to 1.25)
Deerojanawong 2006	0.14 (0.05 to 0.29)	0.15 (0.10 to 0.20)	0.94 (0.39 to 2.25)	1.01 (0.88 to 1.17)
Myalgia				
Deerojanawong 2006	0.16 (0.05 to 0.33)	0.15 (0.10 to 0.20)	1.08 (0.44 to 2.61)	0.99 (0.86 to 1.14)

Table 3 summarises pooled estimates for sensitivity, specificity and positive and negative likelihood ratios with 95% confidence intervals for cough, wheeze, coryza and crepitations. Figure 3 summarises data from the five studies which reported data on cough(100, 103-106). Figure 4 summarises data from the five studies which reported data on crepitations(100, 102, 104-106). Figure 5 summarises data from the four studies which reported data on coryza(103-106). Figure 6 summarises data from the six studies which reported data on wheeze(100-102, 104-106).

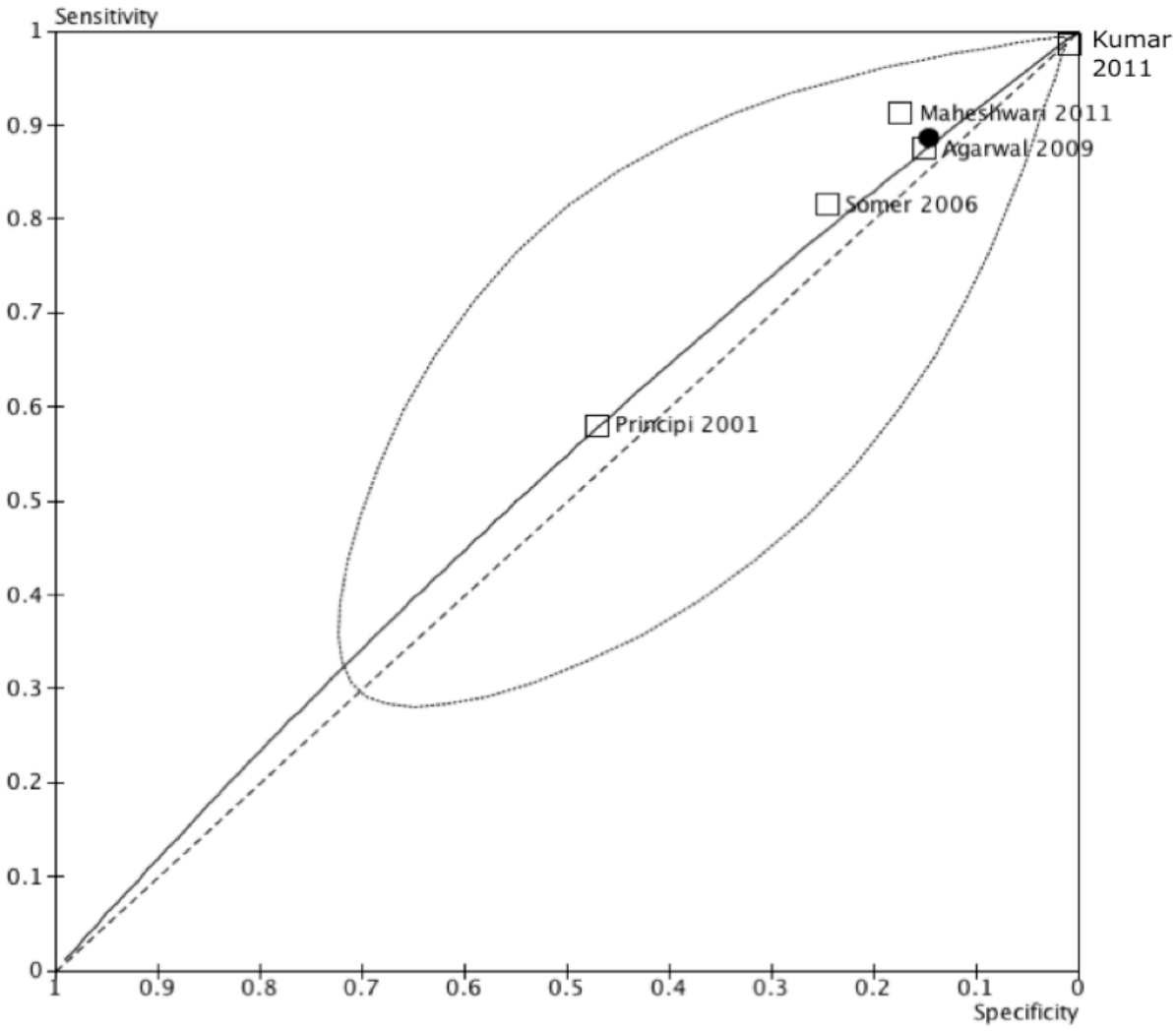
Table 3: Pooled estimates with 95% confidence intervals

Symptom/sign	Number of participants	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR- (95% CI)
Cough*	1076	0.89 (0.67 to 0.97)	0.15 (0.05 to 0.37)	1.04 (0.95 to 1.13)	0.78 (0.44 to 1.39)
Crepitations	1121	0.84 (0.78 to 0.88)	0.22 (0.14 to 0.32)	1.06 (0.96 to 1.18)	0.77 (0.52 to 1.12)
Coryza	833	0.32 (0.08 to 0.72)	0.66 (0.28 to 0.91)	0.95 (0.71 to 1.26)	1.03 (0.90 to 1.17)
Wheeze	1291	0.25 (0.17 to 0.36)	0.67 (0.56 to 0.76)	0.76 (0.60 to 0.97)	1.12 (1.02 to 1.23)

CI = confidence interval; LR+ = Positive Likelihood Ratio; LR- = Negative Likelihood Ratio
*Data on cough from Deerojanawong 2006(102) were not included in this analysis because all participants in the study population had cough.

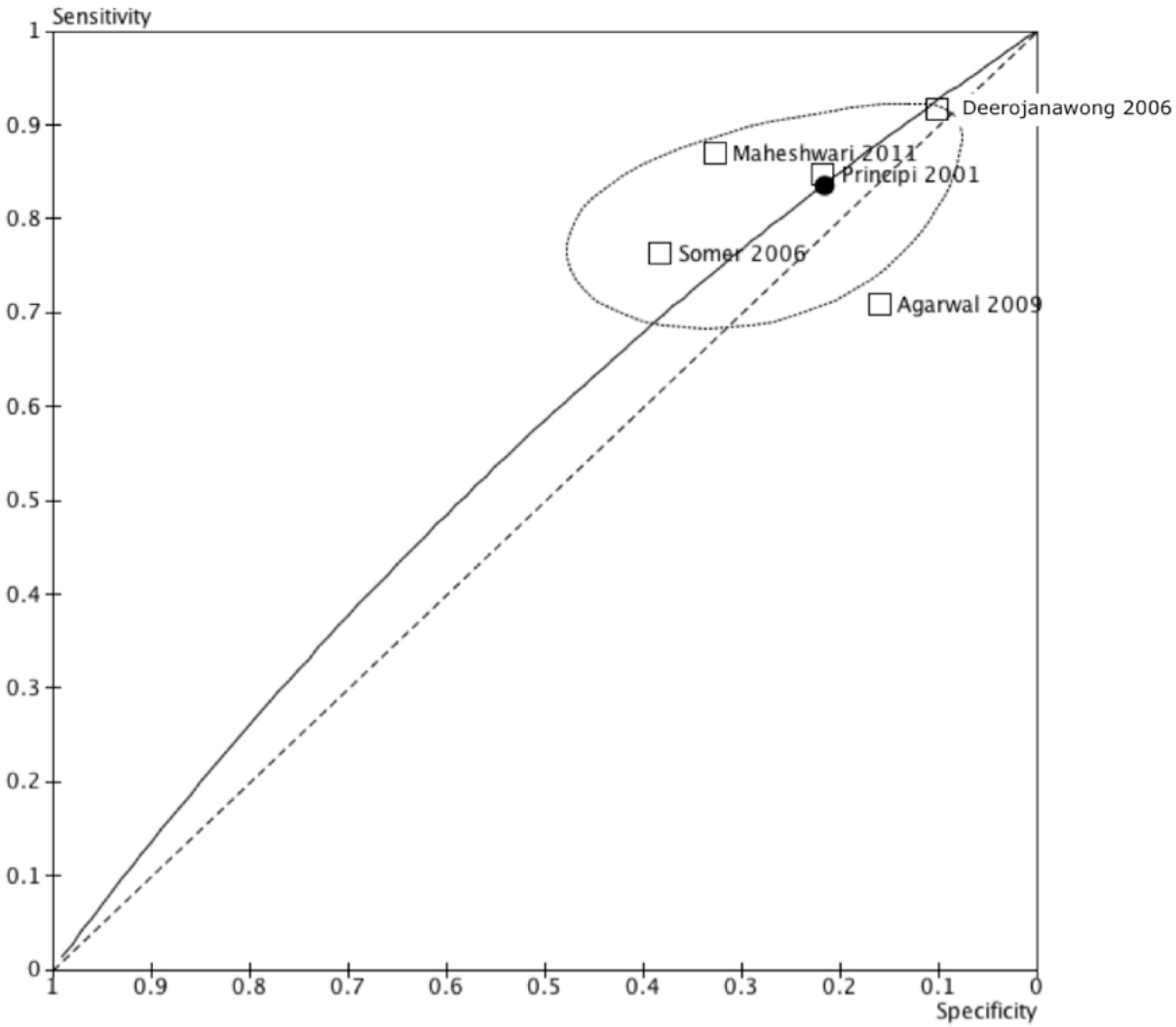
Cough and crepitations were sensitive but poorly specific indicators of Mp. The performance of coryza as a diagnostic indicator was no better than chance (figure 5). Wheeze had poor sensitivity but moderate specificity. The summary curve for wheeze was below the diagonal, indicating that absence of wheeze may indicate Mp infection (figure 6). Although four studies reported data on rhonchi(102-105), the model failed to converge to a summary estimate. This failure to converge could have been due to the small number of studies combined with the data reported for the individual studies and is a well-known problem in these models(107).

Figure 3: Summary Receiver Operating Characteristic Plot of Cough



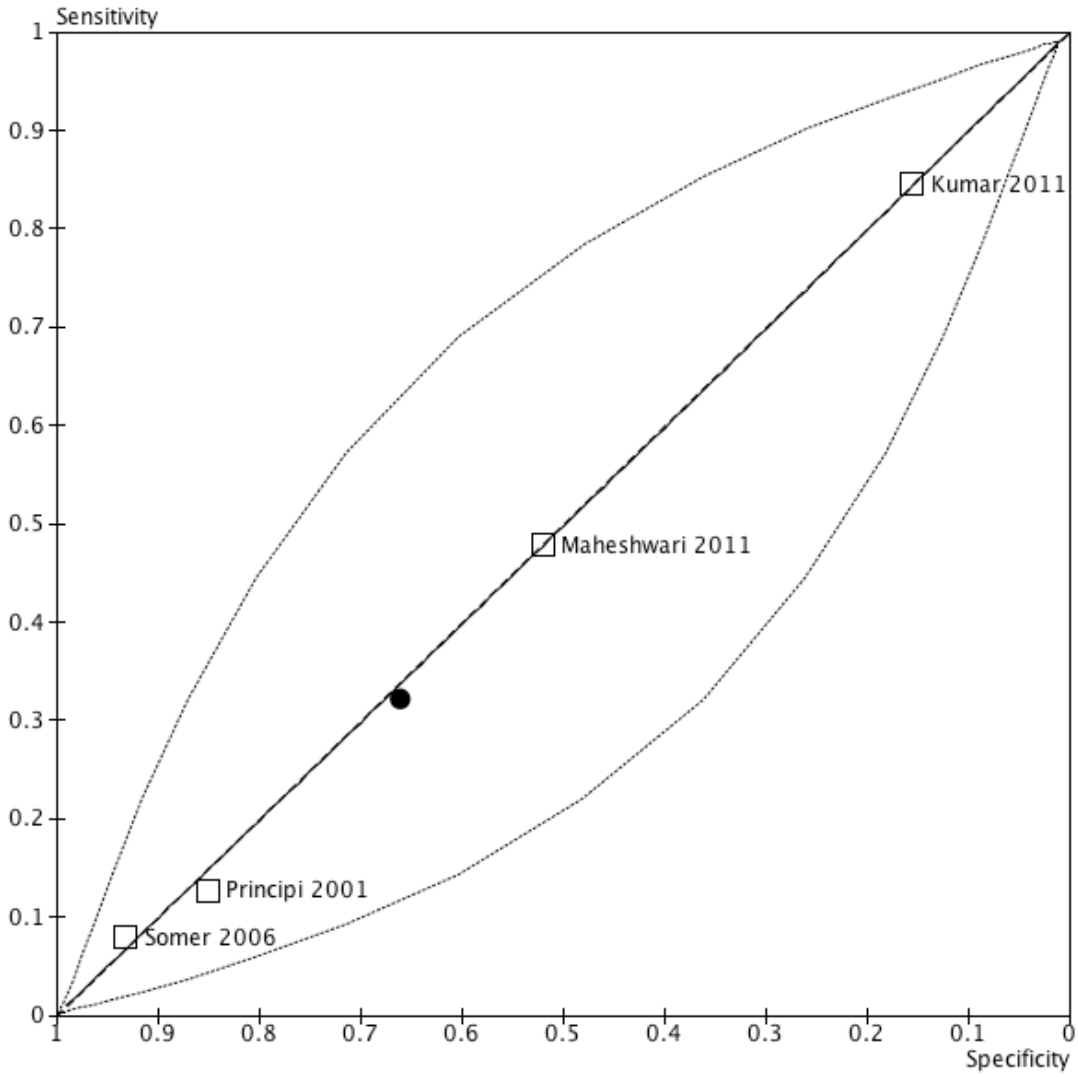
Black square = study (first author, year of publication); Black dot = summary point; Ellipse = 95% confidence region; Black solid line = Summary Receiver Operating Characteristic curve

Figure 4: Summary Receiver Operating Characteristic Plot of Crepitations



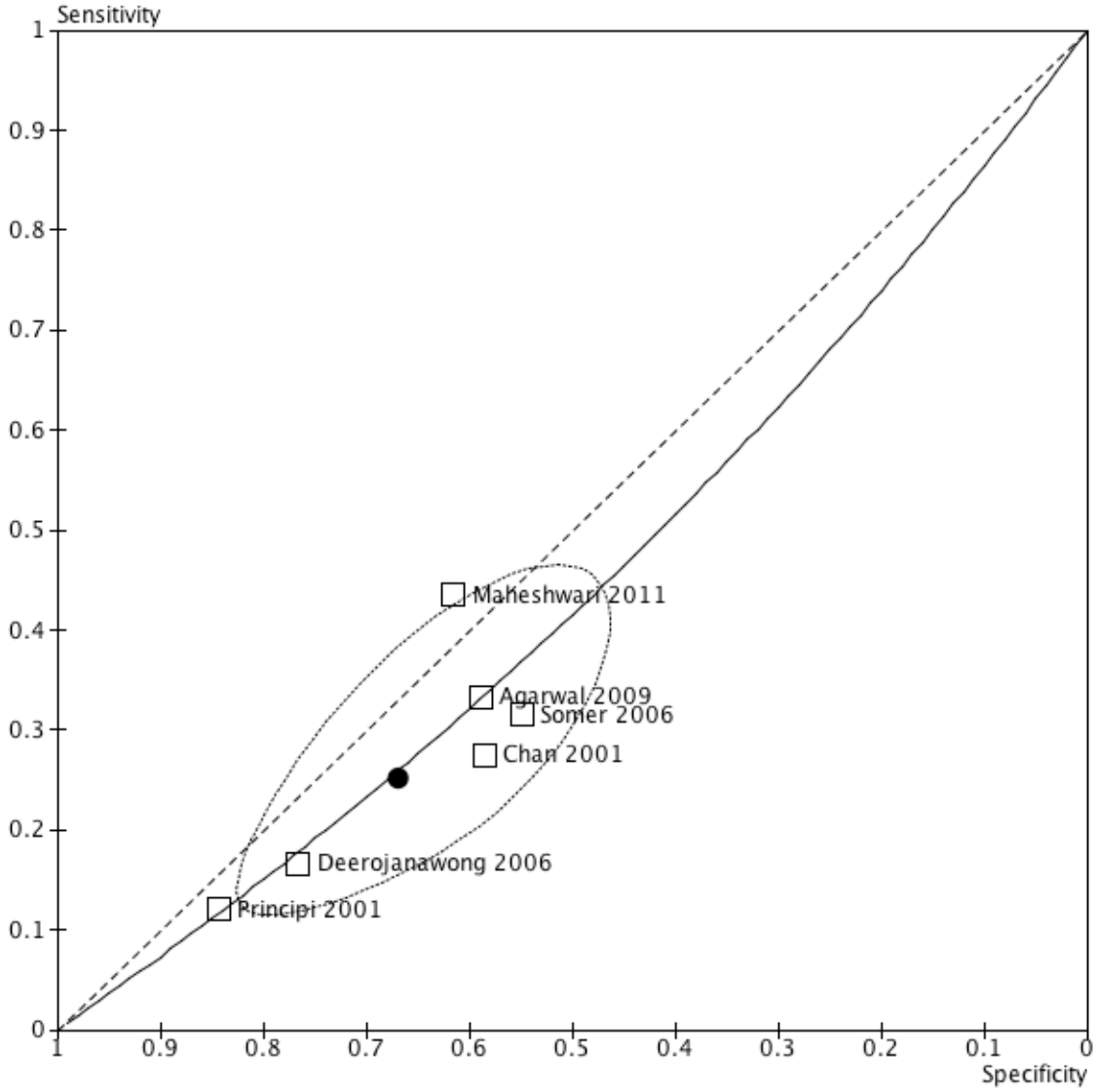
Black square = study (first author, year of publication); Black dot = summary point; Ellipse = 95% confidence region; Black solid line = Summary Receiver Operating Characteristic curve

Figure 5: Summary Receiver Operating Characteristic Plot of Coryza



Black square = study (first author, year of publication); Black dot = summary point; Ellipse = 95% confidence region; Black solid line = Summary Receiver Operating Characteristic curve

Figure 6: Summary Receiver Operating Characteristic Plot of Wheeze

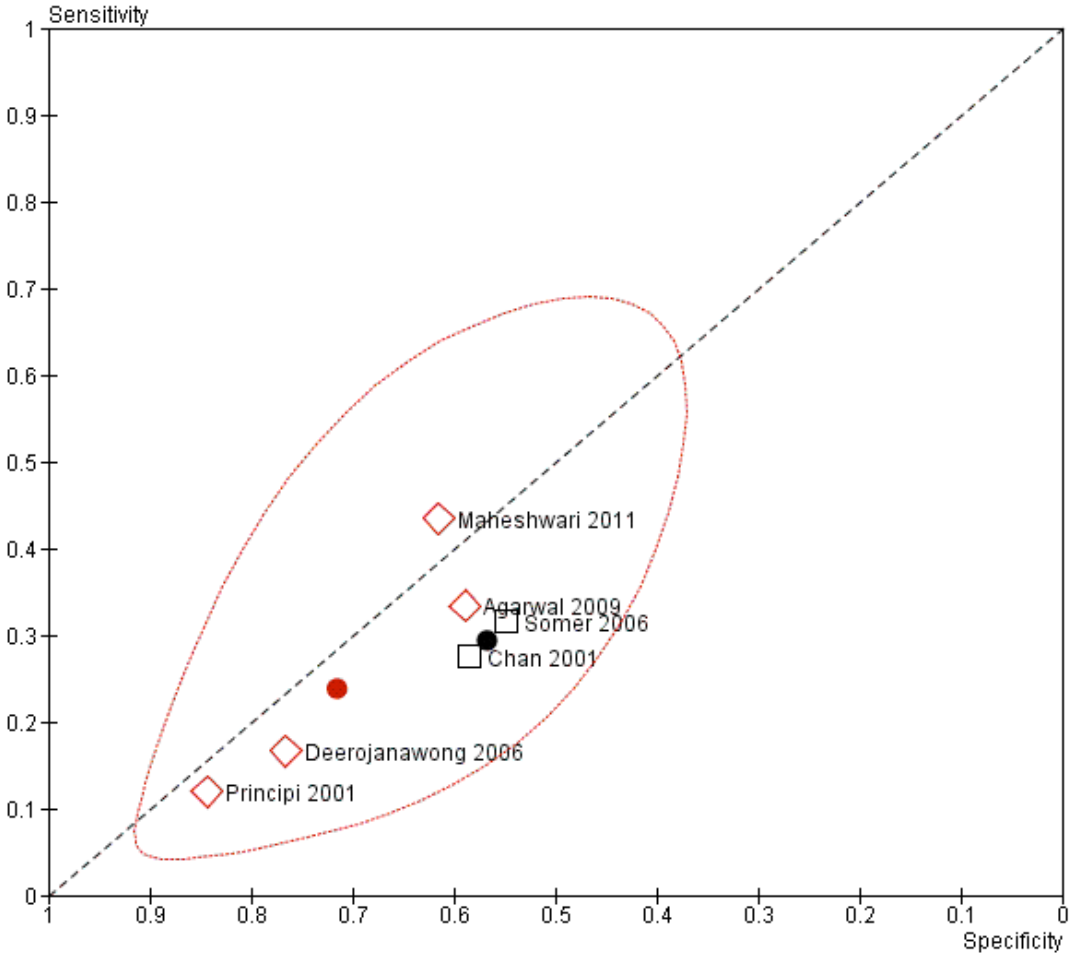


Black square = study (first author, year of publication); Black dot = summary point; Ellipse = 95% confidence region; Black solid line = Summary Receiver Operating Characteristic curve

Wheeze was 12% more likely to be absent in children with Mp (pooled negative likelihood ratio (LR-) 1.12, 95% confidence interval (CI) 1.02 to 1.23). A sensitivity analysis was performed excluding data from Agarwal *et al.* 2009(100) because the review authors considered that this study did not recruit a representative spectrum of patients and had concerns about the acceptability of the reference standard. However, this sensitivity analysis did not change the overall findings (pooled positive likelihood ratio (LR+) 0.75, 95% CI 0.58 to 0.98; pooled LR- 1.11, 95% CI 1.01 to 1.23).

These findings also did not change when data from studies in which Mp was diagnosed using serology only were analysed (pooled LR+ 0.68, 95% CI 0.50 to 0.92; pooled LR- 1.24, 95% 1.03 to 1.51)(101, 106). However, wheeze was not a statistically significant diagnostic indicator based on data from studies which used other laboratory tests alongside serology to diagnose Mp (pooled LR+ 0.84, 95% CI 0.63 to 1.12; pooled LR- 1.06, 95% CI 0.96 to 1.18)(100, 102, 104, 105). Figure 7 summarises individual and pooled sensitivity and specificity values for wheeze based on data from studies which diagnosed Mp using serology only versus other laboratory tests alongside serology.

Figure 7: Summary Receiver Operating Characteristic Plot of Wheeze with summary points for studies using serology only versus serology plus additional laboratory methods to diagnose *Mycoplasma pneumoniae*



Black square = study which diagnosed *Mycoplasma pneumoniae* using serology only; Black dot = summary point for studies which diagnosed *Mycoplasma pneumoniae* using serology only.

Red diamond = study which diagnosed *Mycoplasma pneumoniae* using serology plus additional laboratory methods; Red dot = summary point for studies which diagnosed *Mycoplasma pneumoniae* using serology plus additional laboratory methods; Red ellipse = 95% confidence region of summary point for studies which diagnosed *Mycoplasma pneumoniae* using serology plus additional laboratory methods.

The influence of methods of diagnosing CAP or serological methods of diagnosing Mp were not investigated, since only one study reporting data on wheeze diagnosed CAP based on clinical criteria only and diagnosed Mp using a single high antibody titre(100). Data from this study had already been excluded during the sensitivity analysis. Data were not sufficient to perform investigations of heterogeneity for any other clinical symptoms or signs apart from wheeze. It was not possible to explore the influence of healthcare setting or participant age group because all the studies included in this review were conducted in hospital settings and did not report data stratified according to the age groups of interest.

The presence of crepitations was not a statistically significant indicator of Mp (pooled LR+ 1.06, 95% CI 0.96 to 1.18; pooled LR- 0.77, 95% CI 0.52 to 1.12). However, a sensitivity analysis excluding data from Agarwal *et al.* 2009(100) found that the presence of crepitations was a diagnostic indicator of Mp, although this finding was only of borderline statistical significance (pooled LR+ 1.10, 95% CI 0.99 to 1.23; pooled LR- 0.66, 95% CI 0.46 to 0.96).

Five studies reported data on fever(100, 102, 103, 105, 106). However, a bivariate model could only be fitted when a sensitivity analysis was performed excluding data from Agarwal *et al.* 2009(100). The model did not converge when all five studies were included. Fever had high sensitivity (pooled sensitivity 0.85, 95% CI 0.63 to 0.95) but poor specificity (pooled specificity 0.15, 95% CI 0.05 to 0.38). Overall, fever was not a useful diagnostic indicator (pooled LR+ 1.00, 95% CI 0.94 to 1.07; pooled LR- 1.00, 95% CI 0.70 to 1.44).

Coryza and cough were not useful diagnostic indicators of Mp (Coryza: pooled LR+ 0.95, 95% CI 0.71 to 1.26; pooled LR- 1.03, 95% CI 0.90 to 1.17. Cough: pooled LR+ 1.04,

95% CI 0.95 to 1.13; pooled LR- 0.78, 95% CI 0.44 to 1.39). I attempted a sensitivity analysis of data on cough excluding Agarwal *et al.* 2009(100) but could not obtain a summary measure from the four remaining studies using the bivariate model as the algorithm failed to converge.

4.4 Discussion

4.4.1 Summary of main findings

There is a paucity of high quality data relating to the diagnostic value of symptoms and signs in the clinical recognition of Mp in children and adolescents with CAP. Based on current published data, the absence or presence of individual clinical symptoms or signs cannot be used to help clinicians accurately diagnose Mp. The absence of wheeze is a statistically significant diagnostic indicator of Mp. However, its clinical utility is limited, since the absence of wheeze is only 12% more likely in children with Mp versus children without Mp. However, this review did find preliminary evidence from two studies to suggest that chest pain approximately doubles the probability of Mp in children with CAP.

4.4.2 Strengths and limitations

This review used a systematic and comprehensive search strategy without any language restrictions. Full-text versions of any articles felt to be potentially relevant were analysed, including studies relating to CAP or respiratory tract infections generally, even if Mp was not mentioned in the title or abstract. Two review authors independently screened abstracts and full-text articles as well as extracted data from and assessed methodological quality of included studies. In order to assess the validity and robustness of the review's

findings, sensitivity analyses excluding data from one study(100) were performed because of concerns about the unrepresentative spectrum of patients and the acceptability of the reference standard.

Since there is currently no 'gold standard' for the laboratory diagnosis of Mp, I also assessed the impact on the review's findings of using other laboratory methods alongside serology to detect Mp. A combination of serological and PCR methods is considered to be optimal for detecting Mp in patients with CAP(108). PCR is more sensitive than serology at detecting Mp during the first two weeks after symptom onset(109). A recent study showed that Mp DNA carriage among asymptomatic individuals is rare (1/428 subjects, 0.2%)(110).

The main limitation in conducting this review was paucity of data. Although I had planned to include studies conducted in any healthcare setting, the systematic literature search only found studies conducted in hospital settings. The findings of this systematic review may also have limited applicability in developed countries because all included studies except one(105) were conducted in developing countries.

Data from included studies were only sufficient to obtain pooled estimates for cough, wheeze, coryza and crepitations; pooled estimates for fever could only be obtained during sensitivity analysis. I was also only able to investigate the use of additional laboratory tests alongside serology as a potential source of heterogeneity for one symptom (wheeze). No studies reported data on combinations of clinical symptoms and signs in children with and without Mp.

There were also inconsistencies in the reporting of clinical symptoms and signs across different studies. Coryzal symptoms were described as coryza(103), rhinorrhoea(104), rhinitis(105) or runny nose(106). Rales rather than crepitations were described in four studies(102, 104-106). Although two studies reported data on chest pain(100, 102), no further details were given about its character. In one study, data on the clinical features of two participants with concurrent Mp and *Chlamydia pneumoniae* infections were not reported separately(106). The data were therefore analysed conservatively, assuming that the clinical features being studied were absent in both participants. No studies explored the clinical implications of concurrent infections in patients with Mp.

4.4.3 Conclusions

This systematic review of published data found that Mp cannot be reliably diagnosed based on the absence or presence of individual clinical symptoms and signs. Absence of wheeze was the only statistically significant diagnostic indicator, but did not have sufficient diagnostic value to guide decisions about empirical macrolide prescribing. Data from two studies suggested that chest pain doubles the probability of Mp, but further data are needed to substantiate this finding. Furthermore, no studies were found which recruited children and adolescents with CAP from primary care settings.

This review highlights the need for further research to examine the clinical features of Mp in primary care populations. Chapter 5 examines the prognostic value of diagnosing Mp and respiratory virus infections in a prospectively recruited cohort of children who presented in primary care with persistent cough between 2001 and 2005(111).

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5 *Mycoplasma pneumoniae* and respiratory virus infections in children with persistent cough: a retrospective analysis

5.1 Introduction

5.1.1 Background and rationale

Chapter 4 demonstrated the need for more studies to examine the clinical characteristics of *Mycoplasma pneumoniae* (Mp) in primary care populations, particularly in relation to concurrent infections. This chapter determines the prognostic value of detecting Mp and respiratory viruses in children who present in primary care with persistent cough.

Most coughs in children are acute self-limiting coughs caused by viral respiratory tract infections (RTIs)(1). However, persistent cough is also common in children(2). Parents of children with persistent cough tend to consult when the child appears distressed or unwell and are therefore likely to find precise diagnostic and prognostic information helpful(3). Children with persistent coughs are sometimes diagnosed with asthma. However, asthma is less likely in children with persistent coughs which are not accompanied by wheeze or other respiratory symptoms(4). Overdiagnosis of asthma and inappropriate use of asthma medications is common in children referred to hospital clinics with persistent cough(5).

Previous studies have detected Mp and respiratory viruses in community populations of children with persistent cough(6-8). However, these studies either involved children who had been recruited into pertussis vaccine trials (6, 8) or estimated the prevalence of Mp and respiratory virus infections without considering the clinical characteristics or prognosis associated with these infections(7).

Between October 2001 and March 2005 a cohort of 179 children who presented with a persistent cough lasting two weeks or longer was recruited from general practices in Oxfordshire(9). Thirty-seven percent of these children had evidence of recent pertussis infection based on blood serology and duration of cough was significantly longer in children with pertussis. Nasopharyngeal aspirates (NPAs) were also obtained from these children, which had not previously been analysed. This chapter retrospectively analysed the blood samples, NPAs and cough duration data from this cohort.

5.1.2 Objectives

1. To assess the prognostic value of diagnosing Mp in children with persistent cough.
2. To determine whether the presence of respiratory viruses prolongs cough in children with Mp or pertussis.

5.2 Methods

5.2.1 Recruitment and study procedures

Between October 2001 and March 2005, the Oxford Childhood Infection Study (OXCIS) prospectively recruited children aged 5 to 16 years who presented to their general practitioner with a cough lasting 14 days or longer from 18 general practices in Oxfordshire. Clinical staff at these practices sought consent to obtain blood and NPA samples from each child. For children who had been coughing for between 14 and 28 days at the time of study entry, consent was sought to obtain a second blood sample four to six weeks after the initial sample(9).

Serum samples were stored at -20°C and NPAs at -80°C until testing was performed at the London Health Protection Agency in 2010. Staff at the London Health Protection Agency Respiratory and Systemic Infections Laboratory performed Mp serology using the Mp Enzyme-linked Immunosorbent Assay (recombinant) IgG/IgM Testkit (Virotech, Genzyme Diagnostics) according to the manufacturers' instructions(10). Samples were deemed positive if the Virotech units were above 11.0, borderline if Virotech units were 9.0 to 11.0 and negative if Virotech units were below 9.0. Laboratory staff also detected Mp by performing polymerase chain reaction (PCR) of NPAs using a validated real-time PCR method which amplifies the P1 encoding gene of Mp(11).

Staff at the London Health Protection Agency Respiratory Virus Unit performed real-time PCR of NPAs to detect influenza A (H1 and H3), influenza B, respiratory syncytial virus A and B, and human metapneumovirus(12-14). NPA samples were screened for human rhinovirus using a real-time one-step reverse transcription-PCR (RT-PCR) reaction which targeted the conserved 5' untranslated region (primer and probe sequences available from the London Health Protection Agency on request).

Children in whom the date of onset of cough could not be established were excluded, as were children with chronic underlying medical conditions, which might have been the cause of their persistent cough (including cystic fibrosis, bronchiectasis and cardiac failure). Children with asthma were excluded if clinicians felt that their persistent cough was asthma-related but not if they felt that the cough was unexplained or triggered by an acute RTI.

Clinical staff obtained detailed medical and cough histories on study entry and performed a standardised clinical examination including temperature, peak expiratory flow rate and height. The parent(s) of each child were asked to complete a cough diary for the duration of the child's cough with input from the child if it was felt that he or she had sufficient understanding of the task to do this reliably. For the first 14 days after study entry parents were asked to record in the diary whether or not the child's cough was present on a daily basis. At the end of each subsequent week they were asked to record whether or not the cough was still present until there had been an absence of cough for two consecutive weeks.

5.2.2 Statistical analysis

I performed the statistical analysis of data for this study using PASW Statistics version 18.0. The baseline characteristics of children whose blood and/or NPA samples were still sufficient for analysis were summarised using percentages for categorical variables and medians and interquartile ranges for continuous variables. Data on infections detected by serology and PCR analysis of NPAs were also summarised and proportions were compared using Fisher's exact test.

The OXCIS statistician had previously calculated estimates of the total duration of cough in each child based on the sum of the duration of cough reported at the time of study entry, the number of days that cough was recorded as being present during the first 14 days after study entry and the number of subsequent weeks for which cough was recorded as being present in the cough diary.

I performed Kaplan-Meier analyses to calculate median total cough duration with 95% confidence intervals in children with different infections. Total cough durations were compared using the log rank statistic. Only children whose blood and NPA samples were both sufficient for laboratory testing and whose Mp serology results were not borderline were included in the cough duration analysis.

5.3 Results

5.3.1 Characteristics of study population

Table 1 summarises the baseline characteristics of children whose blood and/or NPA samples were sufficient for analysis (n=170). Of these, 62 had previously been found to have serologically confirmed pertussis infections; 16/62 children had been prescribed beta-lactams and 8/62 had been prescribed macrolides since their cough started. Forty-one children (24.1%) had asthma of whom five also had eczema and one also had hay fever. One child had cerebral palsy, the main manifestations of which were related to motor function and co-ordination; the condition was therefore not considered to be related to the child's persistent cough. All but four children (166/170, 97.6%) had a temperature of less than 37.5°C and peak flow expiratory rate (PEFR) was at least 80% of predicted in 149/166 children in whom PEFR was measured (89.8%).

Table 1: Patient characteristics, n=170

Characteristic	Median (IQR) or number (%)
Age (years)	8.7 (6.5-12.2)
Cough duration on study entry (days)	33 (24.8-49.5)
Sex (male)	95 (55.9)
Ethnicity (White)	162 (95.3)
Medical conditions: Asthma Eczema Hay fever Diabetes mellitus Cerebral palsy	 41 (24.1) 10 (5.9) 3 (1.8) 2 (1.2) 1 (0.6)
Household smoker	63* (39.1)
Antibiotics**	53 (31.2)
Positive pertussis serology	62 (36.5)
Samples suitable for analysis: Blood and nasopharyngeal aspirate Blood only Nasopharyngeal aspirate only	 136 (80) 15 (8.8) 19 (11.2)

IQR = Interquartile range
*n=161 children for whom data on household smoking status were available.
**Antibiotics prescribed for current persistent cough before study entry.

5.3.2 Detection of *Mycoplasma pneumoniae* infection

Table 2 compares detection of Mp infection based on IgM serology versus PCR. In total, evidence of Mp infection was found in 22/170 children (12.9%, 95% confidence interval (CI) 8.7% to 18.8%). Four of these children had been prescribed beta-lactams and two had been prescribed macrolides since the start of their cough. The prevalence of Mp was not significantly higher in children with asthma (6/41, 14.6%) than in non asthmatic children (16/129, 12.4%; p=0.790).

Mp was detected by both serology and PCR in 8 children, by serology only in 11 children and by PCR only in 3 children. Paired serum samples were sufficient for analysis in 27 children. Of these, both samples were positive for Mp IgM in four children and the second sample only in two children. The NPAs of three children who tested positive for

Mp on serology were not sufficient for analysis. Among children whose blood and NPA samples were both sufficient for analysis (n=136) the annual prevalence of Mp varied between 0% (0/25) in 2004 and 25.5% (14/55) in 2002.

Table 2: Detection of *Mycoplasma pneumoniae*, serology versus PCR (n=136)

	Serology positive*	**Serology negative**	**Serology borderline**
PCR positive	8[a]	2	1
PCR negative	8[b]	114[c]	3[b]

PCR = Polymerase Chain Reaction
*3 children were serology positive for *Mycoplasma pneumoniae* but their NPA samples were insufficient for analysis. These 3 children were therefore not included in this table.
[a]Pertussis serology positive, n=1; rhinovirus, n=1
[b]Pertussis serology positive, n=3; rhinovirus, n=2
[c]Pertussis serology positive, n=43

5.3.3 Detection of respiratory virus infections

Table 3 summarises the detection of respiratory viruses in NPAs. The most commonly detected viruses were human rhinoviruses (24/155, 15.5%), which accounted for three-quarters of respiratory virus infections (24/32). Rhinoviruses were detected in the NPAs of similar proportions of children with and without asthma (asthma: 6/38, 15.8%; no asthma 18/117, 15.4%; p=1.00). Seventeen children with respiratory viruses also had evidence of infection with pertussis or Mp (53.1%). Excluding the four children in whom both pertussis and Mp infections were detected, respiratory viruses were found in a higher proportion of children with pertussis (14/46, 30.4%) than with Mp (3/15, 20%). However, the difference between these two proportions was not statistically significant (p=0.524).

Table 3: Detection of respiratory viruses in nasopharyngeal aspirates (n=155)

Respiratory viruses	Number of children	Percentage (95% CI)
Human rhinoviruses	24[a]	15.5 (10.6 to 22.0)
Influenza AH1 AH3 B	4 1 2 1	2.6 (1.0 to 6.5)
Respiratory Syncytial Virus (RSV) RSV A RSV B	3 1[b] 2[b]	1.9 (0.7 to 5.5)
Human metapneumovirus	1	0.7 (0.1 to 3.7)

CI = Confidence Interval
[a]Pertussis serology positive =12, *Mycoplasma pneumoniae* serology positive = 2, *Mycoplasma pneumoniae* serology and PCR positive = 1. [b]Pertussis serology positive = 1

5.3.4 Duration of cough

Total durations of cough (from time cough started) were almost identical between children with single Mp infections detected by serology (median 39 days, 95% confidence CI 24 to 53 days) and PCR (median 39 days, 95% CI 22 to 56 days). Based on serology, children with single Mp infections had a significantly shorter duration of cough than children with pertussis (median 118 days, 95% CI 82 to 154 days, $p<0.001$) or children in whom neither infection was detected (median 70 days, 95% CI 40 to 101 days, $p=0.001$) (figure 1).

Duration of cough was not significantly longer in children with positive pertussis serology and respiratory virus infections than in children with positive pertussis serology alone (median 154 days, 95% CI 74 to 234 days, $p=0.810$). Only three children had evidence of both Mp and respiratory virus infections (human rhinoviruses). In children with human rhinoviruses who did not have evidence of infection with either Mp or pertussis, median duration of cough was 60 days (range 42 to 72 days).

Figure 1: Duration of cough in children with *Bordetella pertussis*, *Mycoplasma pneumoniae* or neither infection detected on serology.

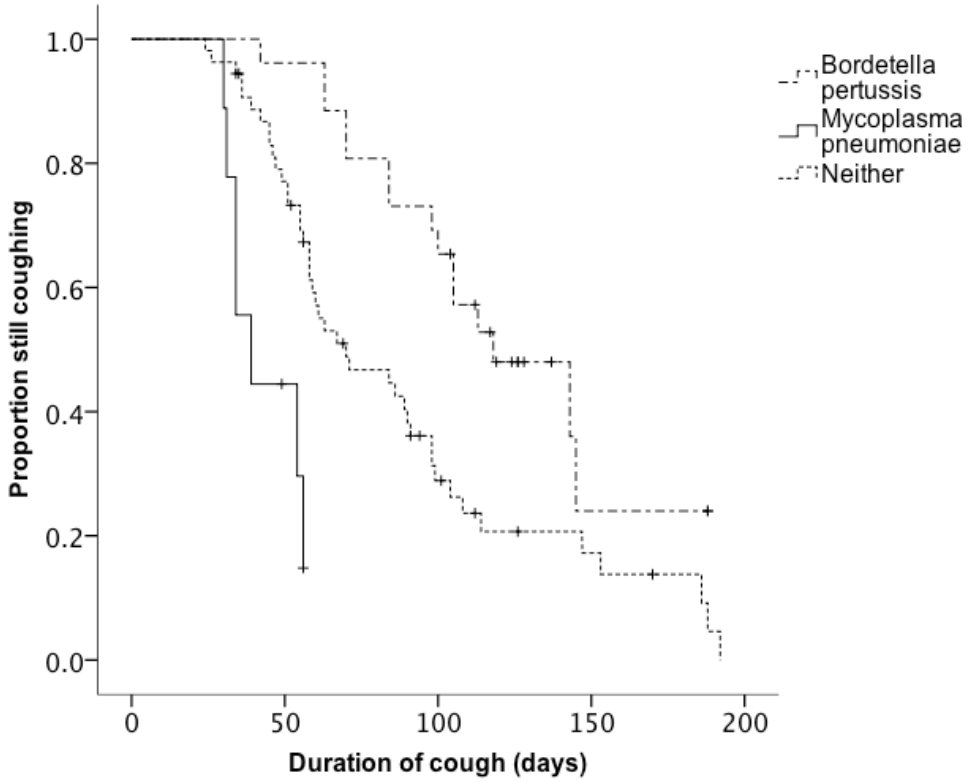

Figure 1: Children with *Mycoplasma pneumoniae* had a significantly shorter duration of cough (median 39 days, 95% confidence interval (CI) 24 to 54 days) than children with pertussis (median 118 days, 95% CI 82 to 154 days, p<0.001) or children in whom neither infection was detected (median 70 days, 95% CI 40 to 101 days, p=0.001). Vertical lines denote censored data. Children whose cough diaries did not indicate that their cough had stopped for at least 2 weeks were censored at the time of the latest diary entry.

5.4 Discussion

5.4.1 Summary of main findings

Although Mp is usually associated with acute RTIs, it may also be found in school-aged children with persistent cough, particularly during periods of high Mp activity. In this sample, Mp was found in one-eighth of children. Early diagnosis of Mp can help reassure children, parents and clinicians that the cough is likely to settle within approximately six

weeks in half of cases. Median duration of cough in children with Mp is only one-third of that in children with pertussis (39 days versus 118 days).

However, establishing the presence of respiratory viruses in children with persistent cough is currently of limited prognostic value. Respiratory viruses, mostly rhinovirus, were detected in one-fifth of children, of whom just over half also had evidence of Mp or pertussis infection. Data were not sufficient to determine the prognostic value of detecting respiratory viruses in children with Mp. However, the additional presence of respiratory virus infections does not significantly prolong cough in children with pertussis.

5.4.2 Strengths and limitations

It was still possible to analyse blood and/or NPA samples from 95% of the originally recruited cohort. However, the quality of samples may have deteriorated in storage over time, which may have reduced detection rates of the infections studied.

For pragmatic reasons, parents were asked, with input from their children if appropriate, to record the absence or presence of cough on a weekly rather than daily basis if the child was still coughing more than 14 days after study entry. This resulted in a high rate of diary completion (119/136, 87.5%) which may not have been achieved with daily diaries since these would have had to be completed for two months or longer from the time of study entry for 37% of children (44/119 completed diaries).

However, daily cough diaries would have improved the accuracy of cough duration estimates. Asking parents to record whether or not the child's cough was present at the

end of each week may have resulted in total duration of cough in some children being underestimated. However, since children in whom the date of onset of cough could not be established were excluded, the maximum magnitude of any underestimation would only have been six days; this is unlikely to have changed the conclusions of this study.

In order to maximise detection of Mp, both PCR and IgM serology tests were used. PCR is superior to serology in detecting Mp in patients with acute respiratory tract infections(15). However, although 11 children tested positive for Mp on IgM serology but not on PCR, positive IgM serology alone was still considered to be a reliable indication of recent Mp infection. A prospective study involving adults with community-acquired pneumonia, which used the same Virotech kit used in this study on single serum samples, reported that IgM antibodies to Mp were very rarely detected in healthy subjects (2/602 blood donors and orthopaedic patients) and that patients who tested positive for Mp on PCR of respiratory samples (sputum or other respiratory secretions) had very similar demographic and clinical characteristics compared to patients with IgM antibodies to Mp only(10). This chapter also demonstrated similar durations of cough in children with single Mp infections based on PCR and serology. This strongly suggests that both methods detected Mp infection accurately. However, IgM serology is of limited use in detecting respiratory viruses as IgM levels are often low due to repeated exposure to circulating virus(16).

Data were not sufficient to allow assessment of whether respiratory viruses prolong cough in children with Mp. Both types of infection were only detected in three children. The high proportion of mixed infections among children with respiratory virus infections suggests that these viruses may predispose children to secondary infections. However, in

this study it was not possible to confirm the timing of different infections in the same child, since sampling was only performed at one time point (study entry).

This study did not perform further investigations to determine the diagnosis of persistent cough in children in whom no infections were detected. However, postinfectious cough is likely to account for a high proportion of these persistent coughs, since the study mostly recruited children who were otherwise healthy and PEFR was at least 80% of predicted in nearly 90% of those in whom this was measured.

This study was unable to assess the effect of macrolides on duration of cough in children with Mp or pertussis. The numbers of children with these infections who had been prescribed macrolides before study entry were small and data on antibiotic prescribing after study entry were not collected.

5.4.3 Conclusions

This chapter demonstrates that the diagnosis of Mp in children with persistent cough has important prognostic value, since duration of cough is significantly shorter in children with Mp than pertussis. However, chapter 4 showed that there is currently insufficient evidence to support the use of symptoms and signs in making a clinical diagnosis.

To facilitate prompt clinical diagnosis of pertussis and Mp in primary care, clinicians should therefore be aware of the prevalence of these infections in the community. Up to date estimates of the prevalence of Mp and pertussis are needed to assess the impact of recent changes in UK childhood vaccination policy, particularly the introduction of the

pre-school pertussis booster vaccination, acellular primary pertussis vaccinations and pneumococcal conjugate vaccine(17).

Chapter 6 estimates the prevalence of pertussis and Mp in children who presented in primary care with persistent cough between November 2010 and March 2012. Chapter 6 also examines baseline patient characteristics as potential predictors of these infections, particularly focusing on previous vaccinations and antibiotic prescriptions.

5.5 References

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6 Prevalence of *Bordetella pertussis* and *Mycoplasma pneumoniae* in children with persistent cough in primary care: a prospective cohort study

6.1 Introduction

6.1.1 Background and rationale

Since the cohort of children discussed in chapter 5 was recruited(1), UK childhood vaccination policy has evolved to include the preschool pertussis booster vaccination (PSB) (2001), acellular primary pertussis vaccinations (aP) (2004) and pneumococcal conjugate vaccine (PCV) (2006)(2). In view of these changes, the present chapter provides updated estimates of the prevalence of *Bordetella pertussis* (pertussis) and *Mycoplasma pneumoniae* (Mp) in school aged children with persistent cough.

The cohort of children discussed in this chapter presented in primary care with persistent cough between November 2010 and March 2012 and includes children who received their childhood vaccinations both before and after the changes in vaccination policy described above. The present cohort therefore provides an ideal opportunity to assess the predictive value of receiving these updated vaccinations in relation to a diagnosis of pertussis or Mp.

This chapter also examines the predictive value of antibiotics prescribed earlier during the persistent cough episode, since previously published data suggest that beta-lactam therapy may increase the likelihood of atypical bacterial infections(3).

6.1.2 Objectives

1. To estimate the current prevalence of pertussis and Mp in children with persistent cough.
2. To assess the value of baseline patient characteristics, previous vaccinations and previous antibiotic use as predictors of pertussis and Mp in children with persistent cough.

6.2 Methods

6.2.1 Recruitment and study procedures

Children aged 5 to 15 years who presented in primary care with an unexplained or postinfectious cough of between two and eight weeks' duration were recruited by healthcare professionals at 15 general practices in Thames Valley. Exclusion criteria were: any serious underlying condition which might be causing the persistent cough (including cystic fibrosis, bronchiectasis or cardiac failure), any known immunodeficiency or immunocompromise or current participation in another clinical research study. Children with asthma were included if their cough was postinfectious but not if it was asthma-related based on their clinician's assessment.

A healthcare professional obtained written informed consent from a parent or guardian for each child to participate in the study. Children were also offered the opportunity to give written informed assent if the healthcare professional recruiting them felt this to be appropriate. The healthcare professional responsible for recruiting the child collected baseline data on date of birth, sex, duration of cough and household smoking status and obtained an oral fluid sample and throat swab from the child.

Staff at the London Health Protection Agency Respiratory and Systemic Infections Laboratory (RSIL) detected evidence of recent pertussis infection using an IgG antigen-capture enzyme-linked immunosorbent assay to detect anti-pertussis toxin IgG (IgG-PT) in oral fluid(4). Oral fluid IgG-PT titres of >=70 arbitrary units (AU) were considered to be consistent with recent pertussis infection in the absence of vaccination less than one year before the date of sampling(5). Oral fluid antibody titres of 60 to 70 AU were considered to be borderline, whilst antibody titres of <60 AU were classified as negative. Staff at the RSIL also detected Mp by polymerase chain reaction (PCR) analysis of throat swabs using a validated real-time PCR method which amplifies the P1 encoding gene(6).

I extracted data from each child's medical record on vaccinations, medical conditions and antibiotics prescribed for the current persistent cough episode before study entry. Ethical approval for this study was granted by Berkshire Research Ethics Committee 10/H0505/44.

6.2.2 Sample size and statistical analysis

I calculated the target sample size and performed the statistical analysis of data for this study. Statistical analyses were performed using PASW Statistics version 18.0.

A target sample size of 300 patients was calculated, which would allow a prevalence of 20% for either pertussis or Mp to be estimated with a precision of +/- 4.5%. However, a sample of 200 patients would give prevalence estimates with a precision of +/- 5.5%.

Baseline participant characteristics were summarised using percentages for categorical variables and medians and interquartile ranges for continuous variables. Estimates of

pertussis and Mp prevalence were calculated with 95% confidence intervals based on the proportion of children with evidence of these infections whose samples were suitable for analysis.

Children who received their PSB less than one year before study entry were excluded from the population in whom pertussis prevalence was estimated. This is because recent vaccination up to one year before sample taking can confound the results of diagnostic serological and oral fluid assays targeting IgG-PT(5).

Logistic regression was used to calculate unadjusted odds ratios with 95% confidence intervals for age, sex, household smoking status and asthma in relation to a diagnosis of pertussis or Mp. Unadjusted odds ratios with 95% confidence intervals were also calculated for receiving beta-lactam and macrolide antibiotics for the persistent cough before study entry in relation to both infections.

In relation to a diagnosis of pertussis, unadjusted and adjusted odds ratios were calculated with 95% confidence intervals for primary pertussis vaccination status, type of primary pertussis vaccine (acellular versus whole cell vaccine), receiving the PSB, type of PSB (five-component versus three-component), duration since receiving the PSB and concomitant primary and booster vaccinations. Concomitant primary vaccinations studied were *Haemophilus influenzae* b (Hib), Meningococcus C (Men C) and Measles, Mumps and Rubella (MMR). Concomitant booster vaccinations studied were Hib and MMR. Children were considered to have received complete primary pertussis vaccinations if they received three doses in total of whole cell and/or acellular primary

pertussis vaccine. Children were categorised as having received the acellular pertussis vaccine if they received one or more doses of acellular primary pertussis vaccine.

Age-adjusted odds ratios were calculated for primary pertussis vaccination status, type of primary pertussis vaccine, receiving the PSB, type of PSB, concomitant primary vaccinations, beta-lactam treatment and macrolide treatment. Odds ratios for concomitant booster vaccinations were adjusted for duration since receiving the PSB. For duration since receiving the PSB, odds ratios were adjusted for type of PSB and type of primary pertussis vaccine.

Children with borderline IgG-PT titres in oral fluid were excluded from the logistic regression analyses. Children whose samples were insufficient for analysis and who had received the PSB less than one year before the date of sampling were also excluded. When calculating odds ratios for receiving the PSB and concomitant primary vaccinations, only children who received complete primary pertussis vaccinations (three doses) were included. When calculating odds ratios for PSB type, duration since receiving the PSB and concomitant booster vaccinations, only children who received complete primary pertussis vaccinations and the PSB were included.

In relation to a diagnosis of Mp, unadjusted and age-adjusted odds ratios were calculated with 95% confidence intervals for household smoking status, PCV vaccination status and treatment with beta-lactam or macrolide antibiotics for the persistent cough before study entry.

6.3 Results

6.3.1 Characteristics of study population

Between November 2010 and March 2012, 184 children were recruited from 15 general practices in Thames Valley. Table 1 summarises the baseline characteristics of these children. Vaccination records were available for all except two children.

Twenty children had asthma (10.9%) of whom 16 were prescribed one or more inhaled medications (80%). One child was being treated with an inhaled short-acting bronchodilator and inhaled corticosteroids because of recurrent lower respiratory tract infections, but had not been diagnosed with asthma or immunodeficiency.

One hundred and seventy children received complete primary pertussis vaccinations (93.4%) and 142 received the PSB (77.2%). One hundred and thirty-seven children received complete primary pertussis vaccinations and the PSB (75.3%).

Of those children who received complete primary pertussis vaccinations, 101 children received three doses of whole cell pertussis vaccine and 62 children received three doses of acellular pertussis vaccine (Pediacel, n=52; Infanrix-IPV-Hib, n=10). Three children received one dose of whole cell pertussis vaccine and two doses of acellular pertussis vaccine. Four children received two doses of whole cell pertussis vaccine and one dose of acellular pertussis vaccine. Eight children received incomplete primary pertussis vaccinations (one dose, n=6; two doses, n=2). Only four children received no primary pertussis vaccinations.

Of those children who received the PSB, 55 (38.7%) received a five-component pertussis vaccine. The duration of time between administration of the PSB and study entry was 0.2 to 9.2 years. Two children received their PSB less than one year before study entry: one child received the PSB at the age of 5.6 years and the other at the age of 8 years.

Fifty-one children received one or more doses of the pneumococcal conjugate vaccine (PCV) (one dose, n=37; two doses, n=5; three doses, n=9). Thirty children had been prescribed antibiotics during their current persistent cough episode before study entry: 20 were prescribed beta-lactams, 8 were prescribed macrolides and 2 were prescribed both beta-lactam and macrolide antibiotics.

Table 1: Patient characteristics (n=184)

Characteristic	Median (IQR) or number (%)
Age (years)	9.1 (6.8 to 11.9)
Sex (male)	106 (57.6%)
Duration of cough (weeks)	4 (3 to 6)
Smoker(s) in household	31 (16.8%)
Asthma[a]	20 (10.9%)
Inhaled short-acting bronchodilator only	3
Inhaled corticosteroids only	1
Inhaled short-acting bronchodilator and corticosteroids	12[b]
Other medical conditions	18 (9.8%)
Atopic eczema	11[c]
Hay fever	6[c]
Neurological conditions	4[d]
Primary pertussis vaccinations[e]	170 (92.4%)
Acellular pertussis vaccine (>= 1 dose)[f]	69 (40.6%)
Preschool pertussis booster vaccination[e]	142 (77.2%)
Five-component vaccine[g]	55 (38.7%)
Concomitant primary vaccinations[f]	
Hib	153 (90%)
Men C	129 (75.9%)
MMR	165 (97.1%)
Concomitant booster vaccinations[g]	
Hib	40 (28.2%)[h]
MMR	123 (86.6%)
Duration since received preschool pertussis booster vaccination (years)	3.8 (2.6 to 6.4)
Pneumococcal conjugate vaccine (>=1 dose)[e]	51 (28.0%)
Antibiotics prescribed during current cough episode	30 (16.3%)

IQR = Interquartile range

[a] Other medical conditions in children with asthma: atopic eczema, n=4; hay fever, n=1; atopic eczema and hay fever, n=1; cerebral palsy, n=1.

[b] One child also treated with leukotriene receptor antagonist in addition to inhaled bronchodilator and inhaled corticosteroids.

[c] Includes 3 children with both atopic eczema and hay fever.

[d] Autistic spectrum disorder, n=2; epilepsy, n=1; attention deficit hyperactivity disorder, n=1.

[e] n=182 children for whom vaccination records were available.

[f] n=170 children who received complete primary pertussis vaccinations.

[g] n=142 children who received a preschool pertussis booster vaccination.

[h] Infanrix-IPV-Hib, n=34; Repevax plus Hib booster, n=6.

6.3.2 Prevalence of *Bordetella pertussis* and *Mycoplasma pneumoniae*

Of the 184 children recruited, the oral fluid samples of eight children were insufficient for analysis and two children received their PSB less than one year before study entry. Evidence of recent pertussis infection was found in 29 of the remaining 174 children (16.7%, 95% confidence interval (CI) 11.2% to 22.2%). Four children had borderline IgG-PT titres in oral fluid.

Of the 137 children who received complete primary pertussis vaccinations and the PSB, 132 had oral fluid samples which were sufficient for analysis. After also excluding the two children who received their PSB less than one year before study entry, evidence of recent pertussis infection was found in 20/130 children (15.4%, 95% CI 9.2% to 21.6%).

Mp was detected in 29/184 children (15.8%, 95% CI 10.5% to 21.1%). Dual pertussis and Mp infections were only detected in one child.

6.3.3 Predictors of *Bordetella pertussis* and *Mycoplasma pneumoniae*

Table 2 summarises unadjusted and adjusted odds ratios with 95% confidence intervals for predictors of pertussis.

Pertussis was more likely in children who did not receive a complete course of primary pertussis vaccinations, although the statistical significance of this finding was borderline after adjustment for age (Odds Ratio (OR) 0.28, 95% CI 0.07 to 1.02, p=0.054). Among children who received complete primary pertussis vaccinations, receiving the acellular pertussis vaccine did not significantly reduce the likelihood of pertussis. Concomitant

administration of the primary Hib, Men C or MMR primary vaccinations also did not significantly predict pertussis.

Although receiving the PSB was not a statistically significant predictor, the odds of pertussis increased by 30% per year after the PSB was administered (unadjusted OR 1.30, 95% CI 1.03 to 1.63, p=0.025). This finding remained statistically significant after adjustment for type of PSB (adjusted OR 1.31, 95% CI 1.03 to 1.65, p=0.025) but not after adjustment for type of primary pertussis vaccine (adjusted OR 1.24, 95% CI 0.95 to 1.63, p=0.118). Concomitant administration of the Hib or MMR booster vaccinations were not statistically significant predictors of pertussis. Previous beta-lactam or macrolide antibiotic treatment were also not significant predictors of pertussis.

Table 2: Predictors of *Bordetella pertussis*

Predictor	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age	1.12 (0.99 to 1.27)	0.082	-	-
Sex	0.94 (0.42 to 2.10)	0.877	-	-
Smoker(s) in household	0.82 (0.26 to 2.58)	0.736	-	-
Asthma	1.89 (0.62 to 5.74)	0.261	-	-
Primary pertussis vaccinations	0.22 (0.06 to 0.77)	0.020	0.28 (0.07 to 1.02)[a]	0.054
Acellular pertussis vaccine (primary)	0.44 (0.17 to 1.19)	0.107	0.52 (0.16 to 1.75)[a]	0.292
PSB	1.22 (0.38 to 3.86)	0.741	4.08 (0.84 to 19.7)[a]	0.081
PSB type (five component)	2.13 (0.81 to 5.59)	0.124	2.18 (0.81 to 5.85)[a]	0.123
Duration since PSB (years)	1.30 (1.03 to 1.63)	0.025	1.31 (1.03 to 1.65)[b]	0.025
Concomitant vaccinations:				
Hib (primary)	0.63 (0.16 to 2.45)	0.507	0.77 (0.19 to 3.12)[a]	0.715
Men C (primary)	0.77 (0.29 to 2.02)	0.595	1.72 (0.39 to 7.58)[a]	0.471
MMR (primary)	0.42 (0.08 to 2.33)	0.323	0.41 (0.07 to 2.28)[a]	0.307
Hib (booster)	0.86 (0.29 to 2.56)	0.780	1.62 (0.45 to 5.92)[c]	0.463
MMR (booster)	0.51 (0.15 to 1.76)	0.284	0.60 (0.17 to 2.16)[c]	0.430
Antibiotics:				
Beta lactam	1.09 (0.34 to 3.51)	0.881	1.09 (0.34 to 3.53)[a]	0.888
Macrolide	1.42 (0.28 to 7.20)	0.674	1.39 (0.27 to 7.15)[a]	0.694

OR = odds ratio; CI = confidence interval; PSB = preschool pertussis booster vaccination; Hib = *Haemophilus influenzae* b; Men C = Meningococcus C; MMR = Measles, Mumps and Rubella
[a]Adjusted for age.
[b]Adjusted for type of PSB.
[c]Adjusted for duration since received PSB.

Table 3 summarises unadjusted and adjusted odds ratios with 95% confidence intervals for predictors of Mp. Mp was more likely in younger children (OR 0.80, 95% CI 0.69 to 0.94, p=0.005) and in children who lived in the same household as a smoker (unadjusted OR 4.13, 95% CI 1.70 to 9.99, p=0.002). The latter remained statistically significant after adjustment for age (adjusted OR 4.80, 95% CI 1.88 to 12.3, p=0.001). Children who received one or more doses of PCV were more likely to have Mp (unadjusted OR 2.46,

95% 1.09 to 5.58, p = 0.031). However, this finding was no longer statistically significant after adjustment for age (adjusted OR 1.02, 95% CI 0.34 to 3.03, p = 0.978). Previous treatment with a beta-lactam or macrolide antibiotic did not significantly predict a diagnosis of Mp. It was not possible to calculate an odds ratio for asthma because none of the children diagnosed with Mp had asthma.

Table 3: Predictors of *Mycoplasma pneumoniae*

Predictor	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age	0.80 (0.69 to 0.94)	0.005	-	-
Sex	1.78 (0.76 to 4.16)	0.181	-	-
Smoker(s) in household	4.13 (1.70 to 9.99)	0.002	4.80 (1.88 to 12.3)*	0.001
Pneumococcal conjugate vaccine	2.46 (1.09 to 5.58)	0.031	1.02 (0.34 to 3.03)*	0.978
Antibiotics:				
Beta-lactam	1.22 (0.38 to 3.90)	0.740	1.26 (0.38 to 4.18)*	0.709
Macrolide	0.58 (0.07 to 4.76)	0.611	0.62 (0.07 to 5.28)*	0.665

OR = odds ratio
CI = confidence interval
*Adjusted for age

6.4 Discussion

6.4.1 Summary of main findings

This chapter estimates that the prevalence of pertussis in school-aged children with persistent cough is 17% and the prevalence of Mp is 16%. Dual pertussis and Mp infections are uncommon.

Pertussis is still prevalent in school aged children with persistent cough despite high coverage with both primary and PSB vaccinations. The odds of pertussis increase by 30% per year after receiving the PSB, even after adjustment for type of PSB.

Clinicians should consider a diagnosis of Mp in younger school aged children with persistent cough who live in the same household as a smoker. Although beta-lactam antibiotics are not recommended for the treatment of Mp, the likelihood of Mp is not significantly increased in children who continue coughing after being prescribed beta-lactam antibiotics.

6.4.2 Strengths and limitations

The sample analysed in this chapter was sufficiently large to estimate a pertussis prevalence of 16.7% with a precision of +/- 5.5% (n=174) and a Mp prevalence of 15.8% with a precision of +/- 5.3% (n=184). Nevertheless, recruitment of this cohort is due to continue until the end of December 2012. Achieving the target sample size of 300 children will allow more precise prevalence estimates. Even if only 240 oral fluid samples are suitable for analysis (*i.e.* 80% retention), this will still enable a pertussis prevalence of 20% to be estimated with a precision of +/- 5%. However, based on the data presented in this chapter, the attrition rate is more likely to be around 5%.

Detection of IgG-PT in oral fluid is a highly reliable method of diagnosing pertussis, with a sensitivity of 93% and a specificity of 94% compared to detection of IgG-PT in serum(4). Based on the prevalence estimate obtained in this study (17%), the oral fluid assay has a positive predictive value of 76% (95% CI 70% to 82%) and a negative predictive value of 98% (95% CI 97% to 99%). Although serology would have given a more accurate prevalence estimate, oral fluid samples are less invasive and technically easier to obtain from children than blood samples. These factors were important in facilitating uptake of the study among children, parents and healthcare professionals.

Based on the results of chapter 5, agreement between serology and PCR of nasopharyngeal aspirates is also high for the diagnosis of Mp (90%). Throat swabs, however, as used in this study, have comparable sensitivity to and are more cost-effective than nasopharyngeal aspirates, since the samples obtained are more likely to be sufficient for analysis(7). However, this study may have missed Mp cases in children who presented at a later stage during their persistent cough. Median duration of persistence of Mp DNA in the upper respiratory tract is reported to be 7 weeks in adults(8) and 5 weeks in children(9) with Mp pneumonia, even after treatment with antibiotics. Bacterial load in children presenting later during their cough may therefore have declined below the diagnostic threshold for PCR. Persistence of Mp DNA is also reported to be longer in children with severe disease (median 6 weeks) than mild disease (4 weeks)(9). Severity of cough was not assessed in this chapter, but nested pilot work measuring 24-hour cough frequency in children with pertussis and/or Mp using the Leicester Cough Monitor(10) is ongoing.

The prevalence estimates obtained in this study would not have been unduly affected by seasonal variation, since recruitment took place over a 17-month period. However, the estimate of Mp prevalence may have been influenced by a period of high Mp activity in England and Wales during late 2011 and early 2012. Between October 2011 and January 2012, PCR was positive for Mp in 9% of community surveillance nose and throat samples obtained from children aged 15 years and under with respiratory symptoms, compared to 1% during the same period in late 2010/early 2011(11). Mp epidemics can last for around 18 months(12). The total 26-month recruitment period of this study (November 2010 to December 2012) will therefore encompass both epidemic and non-epidemic periods and

allow a comparison between endemic and epidemic prevalence of Mp in children with persistent cough.

Data on the proportion of children presenting with persistent cough who participated in this study have not been presented in this chapter. In the cohort studied in chapter 5, from whom blood samples and nasopharyngeal aspirates were obtained, it was estimated from practice audit that 62% of eligible children were recruited(1). However, this proportion may be higher in this study due to the less invasive sampling techniques involved. A similar practice audit after the end of the recruitment period will give an estimate of what proportion of eligible children entered the present study.

Data on baseline clinical characteristics were virtually complete for the cohort studied in this chapter; vaccination records were available for all except two children. These data were therefore sufficient to perform preliminary analyses to identify potential predictors of pertussis or Mp. Odds ratios for individual predictors were adjusted for only one other baseline covariate, since 29 cases each of pertussis and Mp were detected(13). Based on the prevalence estimates obtained so far, the final cohort is likely to include 40 to 50 cases of each of these infections.

In relation to a diagnosis of pertussis, odds ratios for previous antibiotic treatment, primary pertussis vaccination status, type of primary pertussis vaccine and receiving the PSB and concomitant primary and preschool booster vaccinations will be adjusted for household smoking status and asthma in addition to age. The odds ratio for duration since receiving the PSB will be adjusted for type of primary pertussis vaccine in addition to type of PSB. PSB effectiveness will also be estimated; the target sample size of 300

children will allow a PSB effectiveness of 80% to be estimated with an approximate precision of +/- 5%, assuming vaccination coverage of 80%(14).

In relation to a diagnosis of Mp, the odds ratio for household smoking status will, if possible, be adjusted for asthma in addition to age. Odds ratios for previous antibiotic treatment and receiving the PCV will also be adjusted for household smoking status and, if possible, asthma in addition to age. However, assessment of receiving the PCV as a potential predictor may be limited by its recent introduction and therefore relatively low population coverage, particularly in terms of children receiving a complete set of vaccinations (three doses).

6.4.3 Conclusions

Mp or pertussis can be found in around one-third of children with persistent cough, with minimal overlap between these two infections. Living in the same household as a smoker is a potential predictor of Mp, particularly in younger school aged children. The odds of pertussis increase by 30% per year after receiving the PSB, suggesting evidence of waning immunity following vaccination. Pertussis should therefore also be considered in adolescents and adults with persistent cough.

There are currently no proven effective treatments for pertussis-induced or other postinfectious persistent coughs. Chapter 7 is a double-blind randomised placebo-controlled trial whose main objectives are to determine the effectiveness of montelukast for the treatment of postinfectious persistent cough in young people and adults and to collect preliminary data on the efficacy of montelukast in patients whose persistent cough is due to pertussis.

6.5 References

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7 Montelukast for the treatment of persistent cough in young people and adults: a double-blind randomised placebo-controlled trial

7.1 Introduction

7.1.1 Background and rationale

Chapter 6 demonstrated that pertussis is still prevalent among school aged children with persistent cough and that the efficacy of the pre-school pertussis booster vaccination appears to wane with time. Pertussis is therefore likely to also be an important cause of persistent cough in adolescents and adults. However, there are currently no proven effective treatments for pertussis-induced cough(1). Antibiotics are recommended for reducing the risk of transmission, but have not been shown to be effective for treating the cough itself(2).

The present chapter is a double-blind randomised placebo-controlled trial to determine whether montelukast is an effective treatment for persistent cough in young people and adults. Postinfectious cough accounts for nearly half of cases of acute persistent cough of three to eight weeks' duration in non-smoking adults(3), although the preceding acute respiratory tract infection may be subclinical(4). Pertussis is also a major cause of acute persistent cough. A previous study found that 20% of patients aged 12 years and over who presented in primary care over a 15-month period with a persistent cough of one to eight weeks' duration had laboratory evidence of pertussis infection based on culture, polymerase chain reaction (PCR) and/or serology(5).

Montelukast is a leukotriene receptor antagonist, which is reported to improve cough in patients with cough-variant asthma(6). This trial is a proof of principle study based on experimental evidence, which suggests that leukotrienes are also involved in the pathogenesis of postinfectious cough. Increased concentrations of leukotrienes have been observed in the lung tissues of mice infected with Respiratory Syncytial Virus (RSV)(7). In addition, increased production of interferon-gamma (IFN-γ) and leukotrienes has been demonstrated in children with virus-induced wheezing(8). IFN-γ increases the expression of leukotriene receptors in human airway smooth muscle cells *in vitro*(9) and may therefore also potentiate airway inflammation and bronchial hyperresponsiveness *in vivo*.

Leukotrienes may also play an important role in mediating pertussis-induced cough. Pertussis toxin is reported to promote airway hyperresponsiveness by upregulating expression of the leukotriene B4 receptor and interleukin (IL)-1β, both of which are linked to increased synthesis of leukotrienes and prostaglandins(10). A study using the mouse model showed that mice vaccinated with acellular pertussis vaccine and then exposed to pertussis developed lung pathology (peribronchiolitis, perivasculitis, alveolitis and bronchiolar mucus cell hypertrophy) and markers of airway hypersensitivity (increased eosinophil count in bronchoalveolar lavage fluid and increased total serum IgE levels) with similar characteristics to those observed in asthma(11). This study also demonstrated increased *ex vivo* production of pro-inflammatory cytokines (IL-4, IL-5, IL-10 and IL-13) by bronchial lymph node cells(11). IL-13 upregulates leukotriene production and leukotriene receptor expression(12). The inhibitory effect of montelukast on leukotriene-driven inflammation seen in asthma may therefore also reduce persistent cough due to pertussis infection.

7.1.2 Objectives

Primary objective

- To assess the effectiveness of montelukast in the treatment of acute persistent cough in young people and adults.

Secondary objectives

- To assess the adverse event profile of montelukast in the treatment of acute persistent cough in young people and adults.
- To assess the feasibility and practicalities of performing a further trial to determine the efficacy of montelukast in the treatment of acute persistent cough in young people and adults with pertussis.

7.2 Participants and trial design

7.2.1 Participants

Between April 2011 and September 2012, healthcare professionals at 25 general practices in Thames Valley and South West England recruited patients aged between 16 and 49 years who presented with an unexplained or postinfectious acute persistent cough of two to eight weeks' duration. Although the duration of acute persistent cough has previously been defined as three to eight weeks(13), the World Health Organisation's clinical case definition of pertussis includes patients with a persistent cough of two weeks' duration or longer(14). Patients with asthma were included if their cough was postinfectious but not if it was asthma-related based on their clinician's assessment.

Exclusion criteria were: contraindication to montelukast, chronic severe disease which may have been causing the persistent cough (*e.g.* cystic fibrosis, bronchiectasis, cardiac

failure), known immunodeficiency or immunocompromise, current smoker or recent ex-smoker (*i.e.* gave up smoking less than 6 months ago), pregnancy, breastfeeding, patients taking any regular medication associated with persistent cough (*e.g.* angiotensin converting enzyme (ACE) inhibitors) and patients taking part in any other clinical research study.

7.2.2 Trial design

For this double-blind randomised placebo-controlled trial, patients were randomised to receive montelukast or image matched placebo tablets (main excipient lactose monohydrate) with a 1:1 allocation within each general practice. An independent statistician randomly allocated study medication to general practices using randomisation generator software and a fixed block size of four. Healthcare professionals at participating general practices randomly allocated medication to participants, and ensured that all four study medication bottles from one block had been dispensed before starting the next block. Participants, healthcare professionals and research staff were blinded to treatment allocation. The independent statistician retained the randomisation schedule.

On study entry (day 0), healthcare professionals recorded a baseline clinical assessment and obtained an oral fluid sample for pertussis testing from each participant. Each participant completed the Leicester Cough Questionnaire (LCQ)(15) at baseline, two weeks and four weeks after study entry. Participants were also asked to complete a daily cough diary from days 0 to 14. Participants were followed up by a healthcare professional in their general practice two weeks after entering the study, when they were given the option of continuing or discontinuing their study medication. Irrespective of

their choice, healthcare professionals followed participants up by telephone four weeks after entering the study.

The study was approved by Southampton and South West Hampshire Research Ethics Committee (Research Ethics Committee reference number 10/H0502/37; EudraCT number 2010-019647-19; ClinicalTrials.gov Identifier NCT01279668).

7.3 Trial procedure

Healthcare professionals screened patients for eligibility to participate in the trial and obtained written informed consent from each participant. Practices were asked to keep a log of all patients screened and recruited.

Health professionals collected baseline data on date of birth, sex, duration of cough, use of self-administered medication for cough, household smoking status, wheeze and nasal symptoms (sneezing, blocked nose, runny nose, itchy nose) from each participant on study entry (day 0). Participants also completed the LCQ on day 0. Healthcare professionals obtained an oral fluid sample from each participant, which was sent to the London Health Protection Agency to be tested for evidence of recent pertussis infection based on an anti-pertussis toxin IgG (IgG-PT) titre of >= 70 arbitrary units (AU)(16).

Healthcare professionals randomly allocated a bottle of study medication containing 28 montelukast 10 mg or placebo tablets to each participant. Participants were asked to take one tablet daily starting from day 1 (*i.e.* the day after study entry). Participants were asked to avoid taking any self-administered medication for their cough while taking their study medication.

Each participant was given a cough diary and asked to record daily from days 0 to 14 overall cough severity on a 100 mm visual analogue scale (VAS) and the number of paroxysms (bouts) of coughing per day. Participants were also asked to record their study medication intake from days 1 to 14. Each participant completed a second LCQ two weeks after entering the study.

At the two week follow-up appointment, a healthcare professional informed the participant of his or her oral fluid test result. The London Health Protection Agency laboratory notified the local Health Protection Unit of any participant with evidence of recent pertussis infection. Participants were also given the option of continuing or discontinuing their study medication. Healthcare professionals counted, recorded and destroyed any unwanted study medication as well as recorded adverse events and self-administered medication for cough which participants reported having taken. Adverse events were reported to the University of Oxford Primary Care Clinical Trials Unit (PC-CTU). Healthcare professionals provided participants with a third LCQ to be completed four weeks after study entry.

Whether or not participants elected to continue their study medication at the two week follow-up stage, a healthcare professional contacted them by telephone four weeks after study entry and asked them to complete and return their third LCQ. Healthcare professionals also recorded self-administered medications taken since the two week follow-up appointment and reported adverse events to the PC-CTU. Participants were asked to return their study medication bottles, together with any unused tablets, to their general practice. Healthcare professionals at participating general practices counted,

recorded and destroyed any returned study medication and empty study medication bottles.

I extracted data from each participant's medical record on pertussis vaccinations and medical conditions as well as further interventions for the persistent cough during the period from the start of the cough to four weeks after study entry. Further interventions included consultations (excluding the two and four week follow-up appointments), prescribed medications and investigations.

7.4 Outcome measures

The Leicester Cough Questionnaire (LCQ) is a validated patient-completed cough-specific quality of life measure, which has been shown to be responsive to change(15). The LCQ contains 19 items, which are divided into three domains (physical, psychological and social). Patients are asked to rate each item on a 7-point Likert scale. LCQ domain scores can range from 1 to 7 and total scores from 3 to 21. A higher score indicates a better cough-specific quality of life.

Trial participants completed the LCQ at baseline (day 0), two weeks after study entry and four weeks after study entry. The primary outcome measure was the LCQ total score at two weeks. LCQ physical, psychological and social domain scores were secondary outcome measures. Other secondary efficacy outcome measures were cough visual analogue scale (VAS) scores during the two week period after study entry, paroxysmal cough severity during the two week period after study entry and further interventions for the cough during the four week period after study entry. Healthcare professionals

collected data on adverse events from participants at the two and four week follow-up appointments.

To assess the feasibility and practicalities of performing a trial of montelukast in patients with pertussis, the recruitment rate of participants into the trial was estimated. Retention rates and medication adherence rates at two and four weeks after study entry were also estimated in each treatment arm.

7.5 Sample size and trial populations

The minimal important difference (MID) of the LCQ total score is 1.3 with a standard deviation (SD) of 3.3 in adults with chronic cough(17). The MID is defined as the smallest change which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects or excessive cost, a change in the patient's management(18).

The target total sample size was 288 participants, which included allowance for a 25% dropout rate. The target effective sample size was 216 participants (108 participants per arm of the trial), which would give more than 80% power to detect a change in LCQ total score of 1.3, assuming a standard deviation of 3.3 and two-tailed alpha 0.05.

The intention to treat population included all randomised trial participants who completed the LCQ at baseline. The per protocol population included all randomised trial participants who met the trial eligibility criteria, completed their baseline and two week LCQs, did not take any self-administered medication for cough between baseline and two weeks and took at least 12 tablets of study medication during the first two weeks (*i.e.*

medication adherence of at least 85%). The safety population included all randomised trial participants.

7.6 Statistical analysis

I performed all statistical analyses for this trial apart from the calculation of areas under the curve for overall and paroxysmal cough severity. Data were analysed using SPSS version 20.

The baseline characteristics of all randomised participants were summarised by treatment arm, including age, sex, household smoking status, co-morbidity, duration of cough, pertussis status, pertussis vaccination status, previous interventions for the current persistent cough episode and symptoms associated with cough (wheeze and nasal symptoms). Continuous variables were summarised using means and standard deviations (parametric data) or medians and interquartile ranges (non parametric data). Categorical variables were summarised as numbers and percentages.

Recruitment and retention rates in each treatment arm were estimated with 95% confidence intervals. Recruitment rate was calculated based on the proportion of eligible patients who gave consent to participate in the trial. Retention rate was calculated based on the proportions of patients who completed the LCQ at two weeks and four weeks. Retention rates were compared between the treatment arms using Fisher's exact test.

Medication adherence rates at two weeks and four weeks were summarised using medians and interquartile ranges. Medication adherence rate at two weeks was calculated based on the proportion of tablets the participant should have taken (14 tablets) which the participant recorded as actually having taken in the cough diary. Medication adherence

rate at four weeks was calculated in participants who elected to continue their study medication and who returned empty medication bottles or unused tablets at the four week stage.

If the four week follow-up appointment took place on day 28 of the study or after, medication adherence was calculated as: [(28 – number of tablets returned)/28]*100. If the four week follow-up appointment took place before day 28 of the study, medication adherence was calculated as: [(28 – number of tablets returned)/number of days in trial]*100.

Data on LCQ total and domain scores were analysed in the intention to treat and per protocol populations. In the intention to treat population, LCQ total and domain scores were analysed using an intention to treat analysis with last observation carried forward (LOCF)(19). Unadjusted changes in LCQ total and domain scores between baseline and two weeks and between baseline and four weeks were summarised using means and 95% confidence intervals and compared between the two treatment arms using independent t-tests.

Adjusted mean changes in LCQ total and domain scores between baseline and two weeks with 95% confidence intervals and two-sided p values were calculated using analysis of covariance (ANCOVA)(20). The mean differences in LCQ total and domain score changes were adjusted for baseline scores. The change in LCQ total score from baseline to two weeks was also adjusted for age, sex, atopy, pertussis status, pertussis vaccination status, duration of cough and paroxysmal cough severity at baseline. Data on paroxysmal cough severity were obtained from participants' responses to item 11 of the LCQ at

baseline. The mean differences between the two treatment arms in LCQ total and domain score changes from baseline to two weeks were calculated with 95% confidence intervals.

In the intention to treat population only, data from participants' cough diaries on cough VAS scores and number of paroxysms of cough from days 1 to 14 were analysed. The trial statistician used these data to calculate areas under the curve (AUC) as proxy measures for overall cough severity and paroxysmal cough severity respectively. I fitted ANCOVA models to the AUC adjusted for baseline values at day 0. I compared the proportions of patients who reported adverse events and who underwent further interventions for cough during the four weeks after study entry using chi-squared tests.

7.7 Subgroup analyses

I performed exploratory subgroup analyses of changes in LCQ total scores between baseline and two weeks according to pertussis status (positive or negative), atopy (present or absent) and duration of cough at baseline (<=4 weeks versus >4 weeks). I also calculated p values for the interactions between each subgroup variable and the treatment arm. The above subgroup variables were selected *a priori* based on existing literature suggesting that the efficacy of montelukast may be particularly pronounced in patients with pertussis, atopy or who present within four weeks of the onset of cough.

Montelukast may have greater efficacy in patients with pertussis because the pathophysiological mechanism of pertussis-induced cough may be more similar than that of postinfectious cough to cough-variant asthma. Pulmonary eosinophil counts are increased in mouse models of asthma(7) and pertussis(11) but not in mice infected with RSV(7). In addition, children with postinfectious cough do not have airway eosinophilia,

unlike children with untreated asthma(21). Two recent primary care trials demonstrated that leukotriene receptor antagonists (mainly montelukast) were as effective as inhaled steroids as first-line therapy or long-acting beta-agonists as add-on therapy in adults with asthma(22). The efficacy of montelukast may therefore be greater in patients with asthma and other atopic conditions than in non-atopic patients. Finally, the efficacy of montelukast may be greater in patients who commence treatment within four weeks of the onset of cough, since greater spontaneous resolution of cough is observed after this time point(23).

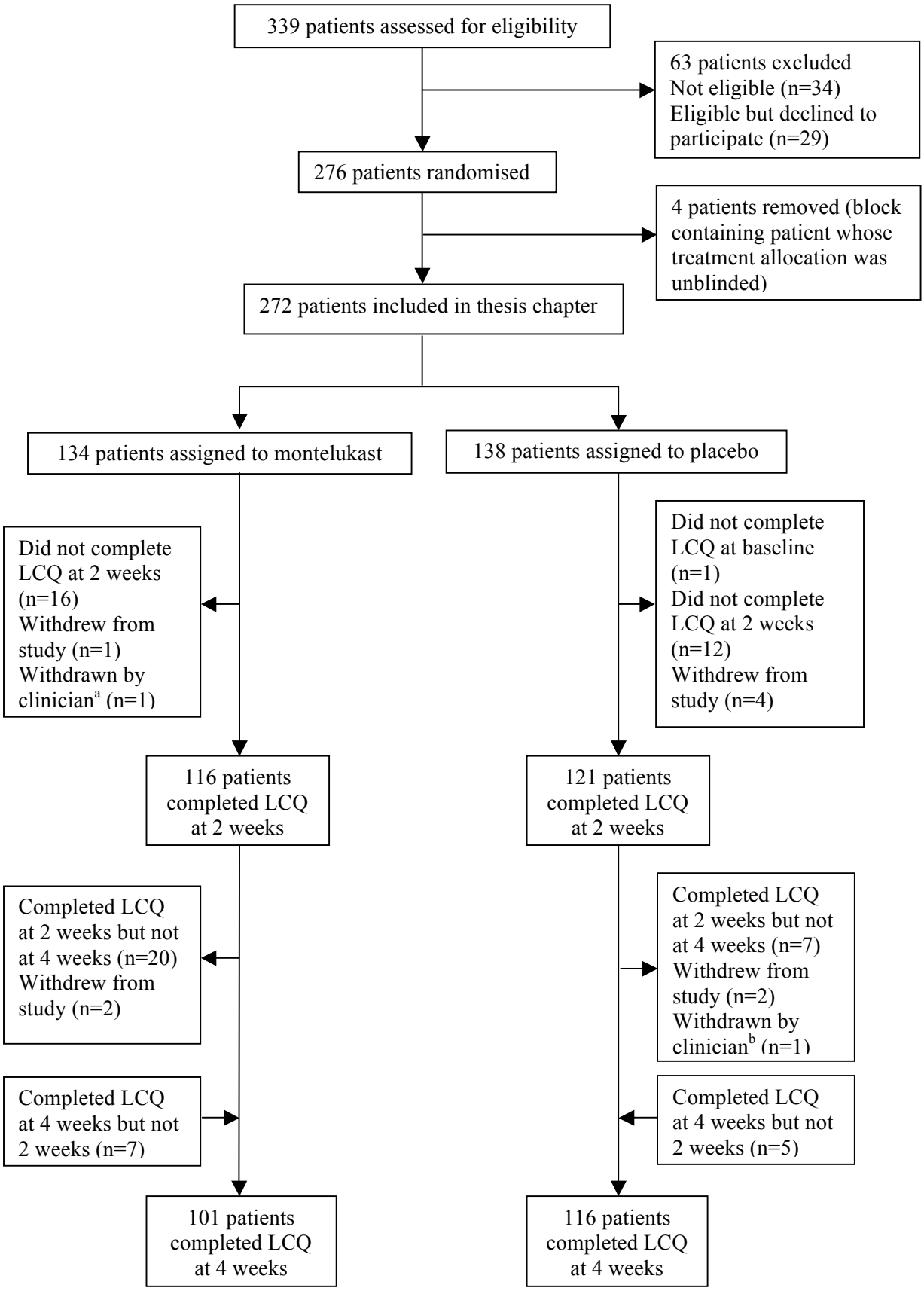
7.8 Results

7.8.1 Recruitment and retention

The results presented in this chapter are based on the data available on 9th November 2012. Between April 2011 and September 2012, 339 patients who presented in primary care with an unexplained or postinfectious persistent cough were screened of whom 276 were recruited into the trial. Eight patients were randomised in error: five patients were over 49 years of age (montelukast, n=3; placebo, n=2), one patient was on ACE inhibitor therapy (montelukast), one patient had previously had a splenectomy (montelukast) and one patient had given up smoking less than six months before study entry (montelukast). Twenty-nine patients were documented as meeting study eligibility criteria but declined to participate in the trial. The recruitment rate was therefore 90.2% (95% confidence interval (CI): 86.9% to 93.6%) based on the proportion of eligible patients who entered the trial (268/297 patients).

Figure 1 summarises the numbers of patients recruited and followed up. One patient in the placebo group experienced shortness of breath and throat tightness after taking one tablet of study medication. A nurse at the walk-in centre where the patient presented managed these symptoms as a suspected anaphylactic reaction to the study medication and informed me of this by telephone. I discussed this event immediately with the Chief Investigator of the trial, who decided that I should be unblinded to this patient's treatment allocation and inform the patient's general practitioner of the treatment allocation. After further investigation, it became clear that this event was not related to the study medication, since the patient had been experiencing these symptoms even before entering the study. However, to prevent me from becoming inadvertently unblinded during data analysis, this patient and the three other patients allocated study medication from the same randomisation block were removed. The trial statistician, who will remain blinded to all treatment allocations, will perform the final analysis of trial data on the full dataset of 276 patients.

Figure 1: Patient recruitment and follow-up



LCQ: Leicester Cough Questionnaire; [a]Clinician withdrew patient after realising that patient had been randomised in error; [b]Clinician withdrew patient due to misunderstanding of trial protocol.

Of the 272 patients included in this chapter, 134 were allocated to receive montelukast and 138 to receive placebo. One patient in the placebo group did not complete the Leicester Cough Questionnaire (LCQ) at baseline. Excluding this patient, the retention rate at two weeks was 87.5% (95% CI: 83.5% to 91.4%) based on the proportion of patients who completed the two week follow-up LCQ (montelukast: 86.6% (95% CI: 80.8% to 92.3%); placebo: 88.3% (95% CI: 82.9% to 93.7%)). The retention rate at four weeks was 80.1% (95% CI: 75.3% to 84.8%) based on the proportion of patients who completed the four week follow-up LCQ (montelukast: 75.4% (95% CI: 68.1% to 82.7%); placebo: 84.7% (95% CI: 78.6% to 90.7%)). There were no significant differences between the treatment arms in relation to retention rates at two weeks (p=0.716) or four weeks (p=0.068).

7.8.2 Baseline participant characteristics

Table 1 summarises the baseline characteristics of trial participants. Evidence of recent pertussis infection was found in 25% of patients (69/272 patients). Forty-one patients had one or more atopic conditions, most commonly asthma (montelukast, n=11; placebo, n=10) or hay fever (montelukast, n=10; placebo, n=7). The most common non-atopic medical conditions were depression (montelukast, n=12; placebo, n=11) and anxiety (montelukast, n=4; placebo, n=6).

Antibiotics were the most commonly prescribed medications and dextromethorphan was the most common self-administered medication which participants reported having taken for their cough before study entry. Baseline clinical severity of cough was similar between the two treatment groups in terms of LCQ total and domain scores, number of paroxysms of cough on day 0 and cough visual analogue scale (VAS) score on day 0.

The proportion of participants who were female was higher in the placebo group than in the montelukast group (71.0% versus 57.5%, p=0.023).

7.8.3 Adherence to study medication

Median medication adherence at two weeks was 92.9% in the montelukast group (interquartile range (IQR) 78.6% to 100%) and 100% in the placebo group (IQR 85.7% to 100%). Ninety-five patients in the montelukast group (70.9%) and 94 in the placebo group (68.1%) elected to continue their study medication at the two week stage. Eighty-six patients in the montelukast group and 91 patients in the placebo group returned unused study medication or empty medication bottles at the four week stage. Among these patients, median medication adherence at 4 weeks was 100% in both groups (IQR 96.4% to 100%). In the montelukast group, 11 patients reported taking self-administered medication for cough between baseline and two weeks (8.2%) and 5 patients between two weeks and four weeks (3.7%). In the placebo group, 11 patients reported taking self-administered medication for cough between baseline and two weeks (8.0%) and 6 patients between two and four weeks (4.4%).

Table 1: Baseline characteristics of trial participants (n=272)

Patient characteristics	Montelukast (n=134) Number (%), mean (SD) or median (IQR)	Placebo (n=138) Number (%), mean (SD) or median (IQR)
Age (years)	37.5 (9.7)	37.9 (9.4)
Sex (female)	77 (57.5)	98 (71.0)
Smoker(s) in household	16 (11.9)	17 (12.3)
Pertussis	30 (22.4)	39 (28.3)
Pertussis vaccination status (three doses)[a]	57 (67.1)	52 (62.7)
Atopy	22 (16.4)	19 (13.8)
Clinical features		
Duration of cough (weeks)	4.8 (1.8)	5.0 (1.9)
Duration of cough <= 4 weeks	67 (50.0)	62 (44.9)
Nasal symptoms[b]	94 (70.1)	104 (75.4)
Wheeze	83 (61.9)	85 (61.6)
Previous interventions for cough		
Primary care consultations[c]	37 (27.6)	45 (32.6)
Chest radiograph	7 (5.2)	18 (13.0)
Prescribed medication:		
Antibiotics	44 (32.8)	52 (37.7)
Inhaled salbutamol	13 (9.7)	12 (8.7)
Codeine or opiates	5 (3.7)	5 (3.6)
Inhaled corticosteroids	2 (1.5)	3 (2.2)
Oral corticosteroids	2 (1.5)	4 (2.9)
Proton pump inhibitors	2 (1.5)	1 (0.7)
Anthistamines	1 (0.7)	1 (0.7)
Self-administered medication:		
Dextromethorphan	30 (22.4)	31 (22.5)
Codeine/pholcodeine	17 (12.7)	17 (12.3)
Antihistamines	8 (6.0)	7 (5.1)
Outcome variables		
LCQ total score[d]	10.7 (2.8)	10.2 (3.0)
LCQ physical domain score[d]	3.8 (1.0)	3.5 (1.0)
LCQ social domain[d]	3.3 (1.2)	3.2 (1.3)
LCQ psychological domain[d]	3.6 (1.0)	3.5 (1.1)
Cough severity, day 0 (VAS score, mm)[e]	60.0 (36.0-74.0)	60.5 (37.0-74.8)
Number of paroxysms of cough, day 0[f]	10 (4-20)	8 (5-20)

SD = standard deviation; IQR = interquartile range; LCQ = Leicester Cough Questionnaire; VAS = visual analogue scale (100 mm)

[a]Number of childhood vaccination records available: Montelukast, n=85; Placebo, n=83. [b]Itchy nose, runny nose, blocked nose or sneezing. [c]Number of patients who had two or more primary care consultations for their current persistent cough episode before study entry. [d]Montelukast, n=134; Placebo, n=137. [e]Montelukast, n=118; Placebo, n=124. [f]Montelukast, n=113; Placebo, n=112.

7.8.4 Efficacy outcomes

Table 2 summarises unadjusted changes in LCQ total and domain scores between baseline and two weeks and between baseline and four weeks. There were no significant differences between the changes in LCQ total and domain scores in the two treatment groups between baseline and two weeks or between baseline and four weeks.

Table 2: Unadjusted change in Leicester Cough Questionnaire total and domain scores between baseline and two weeks and between baseline and four weeks (intention to treat population)

Baseline to two weeks	Montelukast Mean (95% CI)	Placebo Mean (95% CI)	Difference between means (95% CI)	p value
LCQ total	3.13 (2.47 to 3.79)	3.21 (2.62 to 3.80)	-0.08 (-0.97 to 0.81)	0.857
LCQ physical	0.81 (0.62 to 1.01)	0.92 (0.75 to 1.10)	-0.11 (-0.37 to 0.15)	0.402
LCQ psychological	1.14 (0.90 to 1.37)	1.10 (0.88 to 1.32)	-0.04 (-0.28 to 0.30)	0.247
LCQ social	1.18 (0.91 to 1.45)	1.19 (0.97 to 1.41)	-0.11 (-0.37 to 0.34)	0.953
Baseline to four weeks*				
LCQ total	5.63 (4.84 to 6.41)	5.54 (4.83 to 6.26)	+0.08 (-0.99 to 1.15)	0.879
LCQ physical	1.55 (1.31 to 1.79)	1.70 (1.47 to 1.92)	-0.17 (-0.48 to 0.19)	0.395
LCQ psychological	1.96 (1.68 to 2.24)	1.84 (1.59 to 2.09)	+0.12 (-0.26 to 0.50)	0.539
LCQ social	2.11 (1.81 to 2.41)	2.01 (1.73 to 2.28)	+0.11 (-0.30 to 0.52)	0.600

LCQ = Leicester Cough Questionnaire
CI = Confidence Interval
Number of patients who elected to continue study medication at the two week stage: montelukast group, n=95; placebo group, n=94.

Table 3 summarises changes in LCQ total and domain scores between baseline and two weeks adjusted for baseline scores. Patients in both treatment groups reported improvements in LCQ total and domain scores between baseline and two weeks. There were no significant differences between the changes in LCQ total and domain scores in the two treatment groups. Change in LCQ total score between baseline and two weeks in each treatment arm was also adjusted for age, sex, atopy, pertussis status, pertussis vaccination status, duration of cough at baseline and paroxysmal cough severity in

addition to baseline LCQ total score. There was no significant difference between these adjusted changes in LCQ total scores between the two treatment groups (montelukast: +3.23 (95% CI: 2.64 to 3.82); placebo: +3.18 (95% CI: 2.60 to 3.77), difference between means: 0.05 (-0.79 to 0.89), p=0.907).

Table 3: Change in Leicester Cough Questionnaire total and domain scores between baseline and two weeks adjusted for baseline scores (intention to treat population)

Baseline to two weeks	Montelukast Mean (95% CI)	Placebo Mean (95% CI)	Difference between means (95% CI)	p value
LCQ total	+3.24 (2.64 to 3.83)	+3.11 (2.52 to 3.70)	+0.12 (-0.71 to 0.96)	0.772
LCQ physical	+0.88 (0.71 to 1.05)	+0.86 (0.69 to 1.03)	+0.03 (-0.22 to 0.27)	0.837
LCQ psychological	+1.16 (0.95 to 1.37)	+1.08 (0.87 to 1.29)	+0.09 (-0.21 to 0.39)	0.573
LCQ social	+1.19 (0.96 to 1.43)	+1.18 (0.94 to 1.41)	+0.02 (-0.32 to 0.35)	0.918

LCQ = Leicester Cough Questionnaire
CI = Confidence Interval

The area under the curve (AUC) for cough VAS scores from days 1 to 14 was square root-transformed because these data were weakly positively skewed (figure 2). There was no significant difference in overall cough severity between the two treatment arms (montelukast: 21.6 (95% CI: 20.7 to 22.5); placebo: 22.1 (95% CI: 21.1 to 23.0), p=0.494).

The area under the curve (AUC) for the daily number of paroxysms of cough from days 1 to 14 was log-transformed because these data were strongly positively skewed (figure 3). The number of paroxysms of cough at baseline (day 0) was also log-transformed because there was a curvi-linear relationship between log AUC paroxysms of cough and baseline number of paroxysms of cough. After adjustment for log baseline number of paroxysms of cough, there was no difference in paroxysmal cough severity between the two treatment arms (montelukast = placebo = 1.90, 95% CI 1.85 to 1.96, p=0.998).

Figure 2: Distribution of area under the curve for cough visual analogue scale scores from days 1 to 14

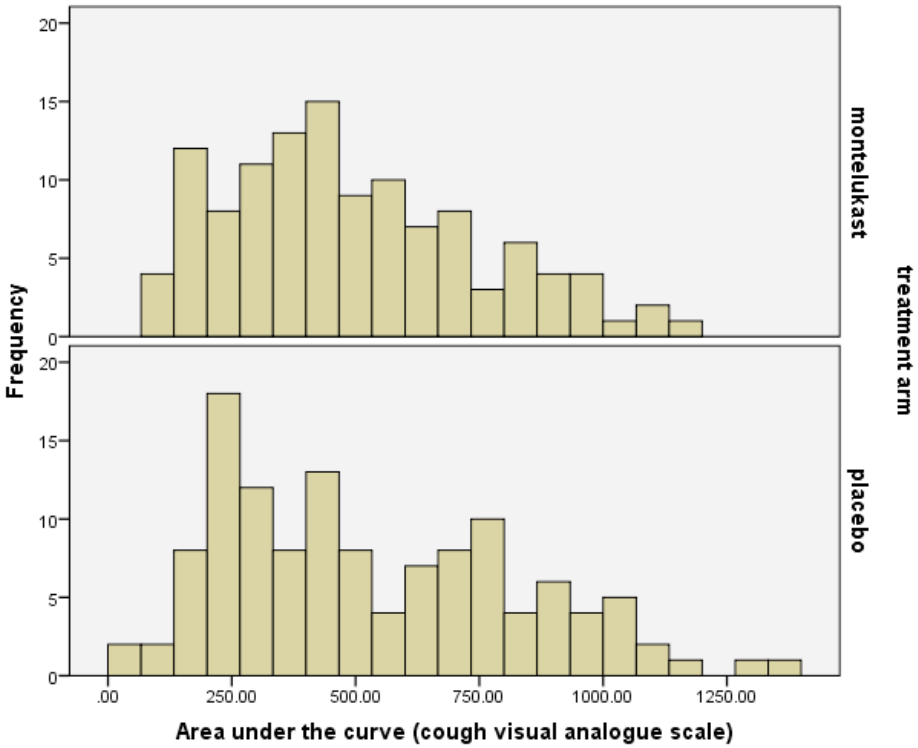
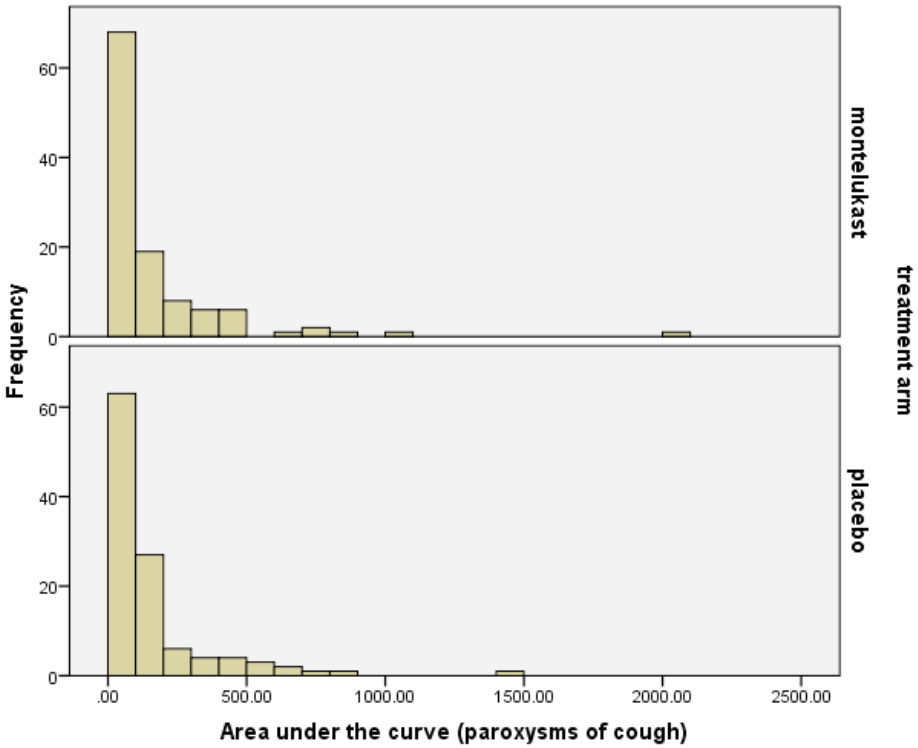


Figure 3: Distribution of area under the curve for daily number of paroxysms of cough from days 1 to 14



The proportions of patients who underwent further interventions for cough during the four weeks after study entry were similar between the two treatment arms (montelukast: 20/134, 14.9%; placebo: 21/138, 15.2%, p=1.000). Table 4 summarises these interventions.

Table 4: Further interventions for cough up to four weeks after study entry

Interventions for cough	Montelukast (n=20) Number (%)	Placebo (n=21) Number (%)
Consultations	11 (55.0%)	10 (47.6%)
Prescribed medication	15 (75.0%)	15 (71.4%)
Chest radiograph	8 (40.0%)	7 (33.3%)
Spirometry	0 (0%)	3 (14.3%)
Serology for atypical pathogens	0 (0%)	1 (4.8%)

n = number of patients who received one or more interventions for cough

7.8.5 Subgroup analyses

Table 5 summarises baseline LCQ total scores and changes in LCQ total scores between baseline and two weeks adjusted for baseline scores according to pertussis status, presence or absence of atopy and duration of cough at baseline (<= 4 weeks or >4 weeks).

Small improvements in LCQ total score changes with montelukast treatment were observed in patients with pertussis (+0.70, 95% CI -0.89 to 2.29, p=0.381), atopy (+0.68, 95% CI -1.32 to 2.68, p=0.496) and a baseline cough duration of four weeks or less (+0.76, 95% CI -0.49 to 2.01, p=0.232). Although none of these treatment effects were statistically significant, the 95% confidence intervals included the minimal and moderate important differences of the change in LCQ total score (1.3 and 1.7 respectively)(17). The 95% confidence intervals of the treatment effects observed in pertussis-negative patients, patients without atopy and patients whose duration of cough at baseline was greater than four weeks did not include the minimal important difference.

No statistically significant interactions were observed between any subgroup variables and the treatment arm (pertussis status, p=0.639; atopy, p=0.300; baseline duration of cough, p=0.175).

Table 5: Changes in Leicester Cough Questionnaire total scores between baseline and two weeks adjusted for baseline scores (subgroup analyses)

	Montelukast		Placebo		Difference between LCQ total changes (95% CI)
	Baseline Mean (SD)	LCQ total change Mean (95% CI)	Baseline Mean (SD)	LCQ total change Mean (95% CI)	
Pertussis					
Positive	10.8 (2.3)	+3.04 (1.87 to 4.20)	9.3 (2.5)	+2.34 (1.32 to 3.35)	+0.70 (-0.89 to 2.29)
Negative	10.7 (3.0)	+3.25 (2.56 to 3.94)	10.6 (3.2)	+3.47 (2.76 to 4.18)	-0.22 (-1.21 to 0.77)
Atopy					
Present	9.9 (2.2)	+3.77 (2.41 to 5.12)	10.7 (3.4)	+3.09 (1.63 to 4.55)	+0.68 (-1.32 to 2.68)
Absent	10.9 (2.9)	+3.13 (2.46 to 3.79)	10.2 (3.0)	+3.12 (2.47 to 3.77)	+0.01 (-0.93 to 0.94)
Duration of cough					
<=4 weeks	10.4 (2.7)	+3.54 (2.67 to 4.40)	9.9 (3.4)	+2.78 (1.88 to 3.68)	+0.76 (-0.49 to 2.01)
>4 weeks	11.1 (2.9)	+2.95 (2.13 to 3.78)	10.5 (2.7)	+3.37 (2.59 to 4.15)	-0.42 (-1.56 to 0.72)

LCQ = Leicester Cough Questionnaire
SD = Standard deviation
CI = Confidence Interval

7.8.6 Adverse events

In the montelukast group, 13 patients reported one adverse event and two patients each reported two adverse events related to study medication. In the placebo group, 15 patients reported one adverse event and one patient reported two adverse events related to study medication. The proportions of patients reporting adverse events related to study medication were similar between the two treatment arms (montelukast: 15/134, 11.2%; placebo: 16/138, 11.6%; p=1.000). Table 6 summarises these adverse events in further detail. Gastrointestinal symptoms (nausea, diarrhoea, bloating) were the most commonly reported adverse events in the montelukast group. Headache, increased mucus production

and skin rashes or itching were the most commonly reported adverse events in the placebo group.

Table 6: Adverse events (safety population)

	Montelukast (n=17)	Placebo(n=17)
Gastrointestinal symptoms	4	2
Headache	3	3
Nasal symptoms	3	1
Rash, itchy skin	2	3
Increased mucus production	1	3
Upper respiratory tract infection	1	1
Other*	3	4

n = number of adverse events related to study medication.
*Montelukast: breast tenderness (n=1); looser cough (n=1); cough triggered by hair and beauty products (n=1). Placebo: pain at top of nose (n=1); painful sinuses (n=1); hiccups (n=1); difficulty sleeping (n=1).

7.8.7 Per protocol analysis

The per protocol population included 87 patients randomised to montelukast and 92 patients randomised to placebo. Table 7 summarises changes in LCQ total and domain scores between baseline and two weeks, unadjusted and adjusted for baseline scores. There were no significant differences between the changes in LCQ total and domain scores in the two treatment groups between baseline and two weeks. Change in LCQ total score between baseline and two weeks in each treatment arm was also adjusted for age, sex, atopy, pertussis status, pertussis vaccination status, duration of cough at baseline and paroxysmal cough severity in addition to baseline LCQ total score. There was no significant difference between these adjusted changes in LCQ total scores between the two treatment groups (montelukast: +3.84 (95% CI: 3.12 to 4.56); placebo: +3.96 (95% CI: 3.25 to 4.67), difference between means: -0.12 (-1.14 to 0.90), p=0.818).

Table 7: Change in Leicester Cough Questionnaire total and domain scores between baseline and two weeks – unadjusted and adjusted for baseline scores (per protocol population)

Unadjusted	Montelukast Mean (95% CI)	Placebo Mean (95% CI)	Difference between means (95% CI)	p value
LCQ total	+3.78 (3.00 to 4.57)	+3.90 (3.16 to 4.64)	-0.12 (-1.21 to 0.97)	0.829
LCQ physical	+0.97 (0.74 to 1.20)	+1.10 (0.88 to 1.32)	-0.12 (-0.45 to 0.20)	0.449
LCQ psychological	+1.36 (1.08 to 1.65)	+1.38 (1.10 to 1.65)	-0.01 (-0.41 to 0.39)	0.995
LCQ social	+1.45 (1.13 to 1.77)	+1.43 (1.14 to 1.72)	+0.02 (-0.42 to 0.45)	0.942
Adjusted for baseline scores				
LCQ total	+3.91 (3.18 to 4.64)	+3.79 (3.08 to 4.49)	+0.12 (-0.90 to 1.14)	0.816
LCQ physical	+1.04 (0.83 to 1.24)	+1.04 (0.84 to 1.24)	0.00 (-0.29 to 0.29)	0.995
LCQ psychological	+1.40 (1.14 to 1.67)	+1.34 (1.08 to 1.60)	+0.06 (-0.31 to 0.43)	0.745
LCQ social	+1.48 (1.18 to 1.77)	+1.40 (1.12 to 1.69)	+0.07 (-0.33 to 0.48)	0.721

LCQ = Leicester Cough Questionnaire
CI = Confidence Interval

7.9 Discussion

7.9.1 Summary of main findings

This trial demonstrates that montelukast is not an effective treatment for acute persistent cough in young people and adults presenting in primary care. Similar improvements in LCQ total and domain scores were observed after two and four weeks in the montelukast and placebo groups. Overall cough severity and paroxysmal cough severity were also similar between the two groups during the two week period after study entry.

However, the findings of our exploratory subgroup analyses suggest that montelukast may be an effective treatment for pertussis-induced cough and postinfectious cough in patients with atopy. Montelukast may also be effective for treating persistent cough in patients who commence treatment within four weeks of the onset of cough. Pertussis-induced cough could be a particularly promising target for montelukast treatment. This trial has

demonstrated that pertussis is prevalent among non-smoking young people and adults with persistent cough and can be found in 25% of these patients.

In addition, this study has established that it would be highly feasible to conduct a double-blind randomised placebo-controlled trial of montelukast in patients with pertussis in primary care. Of those patients who were documented as being eligible to participate, 90% were recruited. Retention rates were over 80% and medication adherence rates over 90% in both arms of the trial after two weeks. Montelukast also has a highly favourable safety profile in patients with acute persistent cough, with similar proportions of patients reporting adverse events related to study medication in both treatment groups.

7.9.2 Strengths and limitations

7.9.2.1 Strengths

This study is one of very few large-scale double-blind randomised placebo-controlled trials conducted in patients with acute persistent cough in primary care. The sample of patients recruited and retained had over 80% power to determine a clinically relevant treatment effect between baseline and two weeks(17) using a validated cough-specific primary outcome measure(15), which was simple and feasible for patients to complete.

Since the items of the LCQ are divided into physical, psychological and social domains, the trial was able to assess the effect of montelukast on these specific areas as well as on overall cough-related quality of life. Patients in both treatment arms were similar at baseline in terms of LCQ total and domain scores, cough VAS scores and daily number of paroxysms of cough. Nevertheless, analyses of these outcomes were adjusted for baseline scores. Although the proportion of female patients was higher in the placebo group than

in the montelukast group, the most clinically relevant outcome (change in LCQ total score between baseline and two weeks) was adjusted for sex.

The main finding of this trial was consistent across multiple cough-related outcome measures in the intention to treat population. The findings in the intention to treat and per protocol populations were also consistent with each other. All exploratory subgroup analyses were specified *a priori* and the treatment effects observed in these subgroups were in accordance with my pre-defined hypotheses. The study also found pertussis to be prevalent in adults with persistent cough (25% of trial participants) and established that a further trial to determine the efficacy of montelukast in patients with pertussis would be feasible and worthwhile.

7.9.2.2 Limitations

This trial aimed to recruit patients with postinfectious and pertussis-induced cough based on eligibility criteria which could be easily assessed by clinicians during routine primary care consultations. While these criteria helped facilitate recruitment, the resulting limitation is that the proportion of trial participants with undiagnosed cough-variant asthma is uncertain. Previous studies diagnosed cough-variant asthma using investigations such as methacholine bronchial provocation testing, capsaicin cough challenge testing and examination of induced sputum(3, 6, 24). However, these are not available in most primary care settings and would therefore not have been feasible to include in the baseline clinical assessment of trial participants.

The data I collected on further investigations and other interventions for cough during the four week period after study entry were not sufficient to determine a definitive diagnosis

for cough in pertussis-negative patients. Although patients were tested for evidence of recent pertussis infection at baseline, the randomisation procedure did not include minimisation for pertussis status. As a result, there was an imbalance between the number of pertussis positive patients in the two treatment arms (30 in the montelukast arm, 39 in the placebo arm). The main limitation of this was in relation to estimating the efficacy of montelukast in the pertussis positive subgroup.

The trial eligibility criteria did not specify a minimum level of cough severity for inclusion, but instead took a pragmatic approach in recruiting patients whose persistent cough was sufficiently troublesome to prompt them to consult. However, this may have resulted in the recruitment of patients with mild to moderate cough, which was already in the process of resolving spontaneously. Indeed, the unadjusted and adjusted mean changes in LCQ total scores from baseline to two weeks in both treatment groups were greater than the large important difference (2.7)(17) in the intention to treat and per protocol populations.

Some trial participants may have consulted due to concerns about the duration rather than severity of cough, especially in view of a national campaign launched in May 2012 to raise awareness of the early signs of lung cancer, including a persistent cough lasting three weeks or longer(25). Baseline LCQ total scores and cough VAS scores in the two treatment arms suggest that cough-related quality of life and overall cough severity were moderate among trial participants. The previously mentioned trials conducted by Price *et al.*(22) specified inclusion cut-offs for scores on the Mini Asthma Quality of Life Questionnaire and Asthma Control Questionnaire, thereby ensuring that only patients with a certain degree of impairment in these measures at baseline were recruited.

For the trial presented in this chapter, setting a LCQ total score cut-off for study inclusion may have helped focus recruitment on patients with more severe cough, who might have gained the greatest benefit from a therapeutic intervention. A comparison of duration of cough between the two treatment arms would also have been worthwhile and informative, but measurement of this outcome would not have been feasible within time-frame available to complete this trial, especially given the protracted time course of pertussis-induced cough(23).

Another limitation of this trial was that it only included subjective measures of cough severity. I attempted to recruit trial participants from five Thames Valley general practices with research nurse support for a pilot study to assess the effect of montelukast on objective cough frequency. This pilot study involved wearing a Leicester Cough Monitor for 24 hours, once at baseline and again after two weeks(26). However, only eight trial participants were willing to participate in this pilot study, hence data were not sufficient to perform any meaningful comparison between the two treatment arms.

Nevertheless, moderate correlation has been demonstrated between 24-hour cough frequency measured using the Leicester Cough Monitor and both LCQ total and cough VAS scores(27). Although I collected data on further interventions for cough as a proxy indicator of persistent or worsening cough, clinicians may have initiated these interventions, particularly further investigations, to rule out serious pathology, irrespective of the severity of the cough(28).

Although the differences in dropout rates after two weeks and four weeks were not statistically significant between the two treatment arms, the dropout rate between baseline and four weeks was almost 10% higher in the montelukast group than in the placebo group. The reason for this is unclear given that adverse event profiles were similar between the two treatment arms.

The trial statistician will examine whether any baseline covariates are significant predictors of dropout using a logistic regression model. If any statistically significant predictors are found, the trial statistician will compute missing LCQ total scores using multiple imputation and then perform an additional sensitivity analysis of the changes in LCQ total scores between baseline and two weeks and between baseline and four weeks. This chapter analysed changes in LCQ total and domain scores using an intention to treat analysis with LOCF, which assumes that missing outcome data are missing not at random (MNAR). However, LOCF was mainly chosen to provide a conservative estimate of the treatment effect, given that acute persistent cough tends to improve rather than worsen over time(19, 23, 29).

This chapter did not assess the effect of the decision to continue or discontinue study medication at the two week stage on the change in LCQ total score between two weeks and four weeks. This will be examined by the trial statistician using a mediation analysis(30).

7.9.3 Conclusions

Montelukast is not an effective treatment for unexplained or postinfectious acute persistent cough. However, montelukast may be an effective treatment for pertussis-

induced persistent cough and postinfectious cough in patients with atopy. Montelukast may also be effective at treating persistent cough if therapy is commenced within four weeks of the onset of cough.

Evidence of recent pertussis infection can be found in 25% of non-smoking young people and adults who present in primary care with acute persistent cough. Based on the rates of recruitment, retention and medication adherence observed in this trial, a further trial of montelukast for the treatment of pertussis-induced cough in primary care would be highly feasible, particularly over a two week treatment period. The next chapter considers other implications for further research and clinical practice, both from this chapter and the other chapters in this thesis.

7.10 References

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8 Conclusions

8.1 Summary of research findings

- *Mycoplasma pneumoniae* (Mp) and pertussis are both prevalent among patients who present with persistent cough in primary care.
- Mp can be found in one-sixth of school aged children with persistent cough. Diagnosis of Mp in these children is of considerable prognostic value, and can help clinicians reassure parents and children that the cough will resolve within six weeks in half of cases.
- Based on currently published data, Mp cannot be reliably diagnosed based on the absence or presence of clinical symptoms and signs. Although the absence of wheeze is a statistically significant indicator of Mp, its diagnostic value is not sufficient to inform decisions about empirical macrolide prescribing. However, living in the same household as a smoker may be a risk factor for Mp in children with persistent cough.
- Evidence of recent pertussis infection can be found in one-sixth of children and one-quarter of adults who present in primary care with persistent cough.
- Although the prevalence of pertussis among school aged children has decreased since the preschool pertussis booster vaccination (PSB) was introduced, the odds of pertussis increase by 30% per year after receiving the PSB, suggesting that immunity wanes with time following vaccination.
- Montelukast is not an effective treatment for persistent cough in young people and adults. However, montelukast may be an effective treatment for pertussis-induced cough and postinfectious cough in patients with atopy. Montelukast may also be an effective treatment for persistent cough if treatment is started within four weeks of the onset of cough.

- A further double-blind randomised placebo-controlled trial to determine the efficacy of montelukast in the treatment of pertussis-induced cough in primary care would be feasible and worthwhile.

8.2 Comparison with existing literature

8.2.1 Clinical diagnosis of *Mycoplasma pneumoniae*

Based on currently published data, chapter 4 found that clinical symptoms and signs were not accurate diagnostic indicators of *Mycoplasma pneumoniae* (Mp) in children with community-acquired pneumonia (CAP). However, this conclusion was based on data collected from children recruited in hospital settings, who are likely to represent a narrower and more severe spectrum of illness than children who present in primary care.

The relationship between illness severity and clinical presentation of Mp is currently uncertain. Greater variation in presenting symptoms and a higher prevalence of certain clinical features (including rhinorrhoea, headache and chest pain) during epidemic outbreaks have previously been observed(1). This may reflect a wider spectrum of disease severity when Mp prevalence is high. In addition, the diagnostic value of clinical symptoms and signs may vary between children of different ages. Two case series found that coryza, tachypnoea, diarrhoea and vomiting were more common in preschool than in older children with Mp(2, 3).

Chapter 4 found preliminary data suggesting that chest pain may be a useful diagnostic indicator of Mp. While there are currently no data on the diagnostic value of chest pain in children with CAP in community settings, a study of adults with CAP found that 41.8% of outpatients reported pleuritic chest pain compared to only 29.3% of inpatients and the

prevalence of Mp was significantly higher in outpatients (29.6%) than in inpatients (6.8%; $p < 0.001$)(4).

The diagnostic value of wheeze may vary according to the prevalence of other respiratory pathogens, particularly respiratory viruses. Chapter 4 may have found that Mp was less likely in children with wheeze because, while the association between respiratory virus infections and acute wheezing episodes is well established(5, 6), Mp and respiratory virus co-infections are infrequent. Evidence of viral infection has previously been detected in 33% of acute asthma exacerbations in children, whereas Mp was only detected in 2%(7). Another study found that Mp was only present in 5% of children with acute wheezing episodes of proven viral aetiology(8). In addition, chapter 5 found that Mp was only present in 3/32 children with persistent cough in whom respiratory viruses were detected (9%).

Chapter 6 found that living in the same household as a smoker was a risk factor for Mp in children with persistent cough. This is consistent with *in vitro* evidence that cigarette smoke exposure impairs Mp clearance in a dose-dependent manner by downregulating expression of a macrophage transmembrane receptor(9). Smoking was also a risk factor for Mp during a disease outbreak in an army training unit(10). However, there was no correlation between the number of cigarettes smoked per day and likelihood of Mp.

8.2.2 Prevalence of *Mycoplasma pneumoniae* in children with persistent cough

In this thesis, the prevalence of Mp in children with persistent cough was estimated to be 7.1% based on Polymerase Chain Reaction (PCR) analysis of nasopharyngeal aspirates (chapter 5) and 15.8% based on PCR of throat swabs (chapter 6). These estimates are

considerably higher than those reported in children with acute cough and fever (2.6%)(11) and asymptomatic household contacts of Mp cases (0.25%)(12). This suggests that the presence of Mp in children with persistent cough represents active infection rather than carriage. However, chapter 5 may have observed a relatively short duration of cough in these children because, since Mp replicates slowly and has limited capacity for protein biosynthesis, it may be more efficiently cleared by the immune system(13).

Detection rates of Mp in children with persistent cough are likely to vary with patient age and timing of recruitment. A two-year prospective study of children with a one to six week history of cough found Mp in only 4.4% of 136 cough episodes based on PCR of nose and throat swabs(14). A prospective cohort study of children referred to a tertiary hospital with a persistent cough of more than three weeks' duration found Mp in only 1.9% of patients based on rising total antibody titres and PCR of bronchoalveolar lavage fluid(15). These studies may have estimated a lower prevalence of Mp in their study populations because participants consisted mainly of preschool and young school-aged children. However, the highest incidence of Mp is found in school aged children, especially those aged 5 to 9 years(16).

Mp prevalence estimates are also likely to be influenced by timing of recruitment in relation to Mp epidemics, which occur at approximately four-yearly intervals(12). The increased prevalence of Mp during 2002 which was observed in chapter 5 was also observed nationally(17). However, the recruitment periods of shorter studies may not coincide with Mp epidemics. Seasonality of recruitment may also influence estimates of Mp prevalence, since Mp occurs with increased frequency during spring and winter in England and Wales(18).

8.2.3 Prevalence of pertussis in children with persistent cough

Chapter 6 demonstrates a considerable reduction in the prevalence of pertussis among school aged children with persistent cough from 37% before the PSB was introduced(19) to 17% following introduction of the PSB. Pertussis nevertheless remains a prevalent cause of persistent cough and becomes increasingly likely with time after receiving the PSB. These findings are consistent with those reported in a study of national pertussis vaccination and surveillance data from Norway between 1996 and 2010, which found that the risk of pertussis was markedly reduced in cohorts with high PSB coverage(20). However, the proportion of pertussis cases in vaccinated individuals increased over time. This suggested that waning immunity rather than increased disease transmission increased the likelihood of pertussis in older school aged children.

Evidence of waning immunity has also been reported by recent studies conducted in the United States. During a pertussis outbreak in California, the rate of laboratory-confirmed pertussis in children between the ages of 8 and 12 years increased in proportion to duration of time since the PSB vaccination(21). Furthermore, a case-control study by Klein *et al.* estimated that the odds of pertussis increased by 42% per year after receiving the PSB(22). This estimate is greater than the corresponding odds ratio reported in chapter 6 (30% per year).

Two main factors may have contributed to this difference. Firstly, Klein *et al.*(22) did not report whether pertussis testing was only performed in patients with clinically suspected pertussis or was also performed in asymptomatic contacts of pertussis cases. If the latter occurred, the odds ratio estimate may have been inflated due to a higher proportion of

symptomatic patients among cases than controls. Such inflation would not have occurred in chapter 6 because all children presented with persistent cough.

The estimate reported by Klein *et al.*(22) may also have been inflated because the study used PCR testing to diagnose pertussis. However, PCR cannot differentiate between viable and dead organisms(23, 24). Pertussis 'cases' may therefore have included patients who did not have active pertussis infection. Diagnosis of active pertussis infection would have been highly accurate in chapter 6, since the detection of anti-pertussis toxin IgG (IgG-PT) in oral fluid is an excellent surrogate for serology(25) and only 0.8% of the general population have been shown to have IgG-PT titres above the diagnostic threshold(26).

In England and Wales, PSB vaccine effectiveness is estimated to be only 46% (95% confidence interval -7% to 71%) in children who received PSB and primary vaccinations versus primary vaccinations only(27). However, this is only based on confirmation of pertussis cases using culture and/or PCR. Culture and PCR may not detect pertussis in patients presenting with a persistent cough of longer than three weeks' duration due to bacterial loads falling below the diagnostic threshold(28, 29). Nevertheless, this finding is consistent with the observation in chapter 6 that PSB vaccination status does not significantly predict a diagnosis of pertussis.

Failure to diagnose cases presenting at a later stage is unlikely to have occurred in chapter 6, since children presented with a cough of two to eight weeks' duration at the time of sampling and longitudinal modelling of IgG-PT levels following clinical pertussis infection suggests that these persist above the diagnostic threshold for a mean of 134 days(26).

Chapter 6 found that type of PSB (three-component versus five-component acellular pertussis vaccine) was not a significant predictor of pertussis. This finding contrasts with the observation that, when previously vaccinated children developed pertussis, antibody responses were more robust against the antigens contained in the vaccine they received(30). This would suggest that pertussis vaccines containing more antigens should have greater efficacy. In addition, a randomised controlled trial reported that a five-component acellular pertussis vaccine had greater efficacy than a three-component vaccine in preventing culture-confirmed pertussis with paroxysmal cough for at least 21 days(31). However, this trial administered three vaccine doses to each participant (3, 5 and 12 months) and had a relatively short duration of follow-up (mean 22 months from third dose).

The effect of primary pertussis vaccination type (acellular or whole cell) on an individual's response to subsequent pertussis booster vaccinations is still uncertain. Enhanced post-PSB T-cell memory responses in relation to pertussis-specific Tumour Necrosis Factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin (IL)-5 production have been reported in children who received whole cell but not acellular pertussis vaccine during infancy(32). In contrast, the functionality of IgG-PT following an adolescent pertussis booster vaccination was found to be greater in patients previously vaccinated with five doses of acellular pertussis vaccine than in patients vaccinated with four doses of whole cell pertussis vaccine(33). In chapter 6, duration since PSB vaccination was no longer a statistically significant predictor of pertussis after adjustment for primary pertussis vaccination type. However, this finding will be re-examined once recruitment of the cohort is complete.

8.2.4 Role of montelukast in the treatment of persistent cough

Chapter 7 found that montelukast was not an effective treatment for acute persistent cough in young people and adults. This contrasts with the results of previous double-blind randomised placebo-controlled trials, which have found leukotriene receptor antagonists to be effective in treating cough in patients with cough-variant asthma. A small crossover trial (n=8) found that zafirlukast improved subjective cough scores and suppressed cough sensitivity to capsaicin compared to placebo in patients with cough-variant asthma refractory to inhaled beta-agonists and corticosteroids(34). Another trial found that a four week treatment course of montelukast 10mg daily reduced cough frequency by 75.7% compared to 20.7% in the placebo group (montelukast, n=8; placebo, n=6)(35).

Montelukast 10mg daily for four weeks significantly decreased cough visual analogue scale (VAS) scores, cough sensitivity to capsaicin and sputum eosinophil counts compared to baseline in non-smoking adults with treatment-naïve cough variant asthma(36). However, pulmonary function, airway responsiveness to methacholine and sputum levels of inflammatory mediators (including cysteinyl leukotrienes and leukotriene B4) did not change.

These findings suggest that the anti-tussive effect of montelukast seen in cough-variant asthma may be due to reductions in cough receptor sensitivity and eosinophil count rather than leukotriene-mediated airway inflammation or hyperresponsiveness. This may explain the results of the exploratory subgroup analyses in chapter 7, which found that montelukast may be an effective treatment for cough in patients with pertussis and atopy. While both these conditions are associated with increased pulmonary eosinophil counts in the mouse model(37, 38), postinfectious cough is not associated with airway

eosinophilia(39). However, increased pulmonary eosinophils have been observed in mice infected with Respiratory Syncytial Virus having previously been sensitised with mite allergen(37), simulating postinfectious cough in patients with asthma.

There is currently scarce published literature on the effectiveness of montelukast in the treatment of unexplained or postinfectious persistent cough. A systematic review of leukotriene receptor antagonists for prolonged non-specific cough in children(40) found only one randomised placebo-controlled trial of montelukast which examined cough-specific outcomes (day time and night time cough)(41). However, data on isolated persistent cough were only available for six participants, of whom five were treated with montelukast and one with placebo.

One trial randomised adults with postinfectious cough to montelukast 10mg four times daily and erythromycin 250mg twice daily (n=45) or ketotifen 1mg four times daily and erythromycin 250mg twice daily (n=44) for 5 days(42). Patients were asked to score cough severity from 0 (no cough) to 3 (severe cough) and rate the degree of improvement in their cough before and after treatment. This trial concluded that montelukast was an effective treatment for postinfectious cough based on a greater reduction in cough scores in the montelukast group (before treatment: 1.89 ± 0.73 (mean ± standard error); after treatment: 0.53 ± 0.19) versus the ketotifen group (before treatment: 1.79 ± 0.80; after treatment: 1.23 ± 0.36) and a higher proportion of patients reporting complete cure or marked improvement in cough in the montelukast group (80%) than in the ketotifen group (48%).

However, the clinical relevance of these treatment effects is uncertain. The outcome measures used were unvalidated and highly subjective, no justification was provided for

the trial sample size and it was not clear whether participants or research staff were blinded to treatment allocation. The trial presented in chapter 7 therefore represents an important contribution to the evidence base for the role of montelukast in the treatment of persistent cough.

8.3 Implications of findings for clinical practice

This thesis demonstrates that diagnosing Mp or pertussis in patients with persistent cough can be of considerable value in guiding clinical management. Clinicians should therefore be aware of which patients are more likely to develop these infections and consider confirmatory testing in patients who do not fit diagnostic criteria for other underlying conditions associated with persistent cough.

Diagnosing Mp in children with persistent cough can help clinicians to reassure parents and children about its relatively short time course and set appropriate thresholds for further investigation and treatment if the cough does not settle within the expected timeframe. Mp may be more likely in younger school aged children with persistent cough who live in the same household as a smoker (chapter 6). In addition, the presence of chest pain or absence of wheeze may suggest Mp in children with community-acquired pneumonia (chapter 4). However, primary care clinicians should interpret the findings of chapter 4 with caution, since they are based on studies which recruited children from hospital rather than primary care settings.

Although the absence of wheeze was a statistically significant diagnostic indicator of Mp, clinicians should not base decisions about empirical macrolide prescribing on this clinical feature. Based on the pooled sensitivity estimate reported in chapter 4, a policy of

empirical macrolide treatment in children without wheeze would result in antibiotics being withheld from 25% of children with Mp. More importantly, a high percentage of children receiving empirical antibiotic treatment would be Mp-negative and therefore receive antibiotics unnecessarily. Given the pooled specificity of 67% reported in chapter 4, the percentage of children receiving antibiotics unnecessarily would range from 61% if Mp prevalence was 36% (the highest prevalence estimate among the studies included in chapter 4) to 89% if Mp prevalence was 10% (the lowest prevalence estimate among the included studies). Indeed, clinicians should carefully consider population-level disease data when making decisions about empirical macrolide prescribing, given the cyclical epidemic nature of Mp.

Diagnosing pertussis in patients with persistent cough in primary care is important to ensure that prompt measures are taken to reduce disease transmission. To decrease the duration of the infectious period, treatment with macrolide antibiotics (or co-trimoxazole if macrolides are contraindicated) is recommended in confirmed or clinically suspected pertussis cases if diagnosed within 21 days of the onset of cough(43). Chapter 6 demonstrates that pertussis is still prevalent among children with persistent cough despite high primary and PSB vaccination coverage. Clinicians should therefore still consider pertussis in patients with persistent cough who have received both primary and PSB vaccinations. Clinicians should have a particularly high index of suspicion for pertussis in adolescents and adults with persistent cough, since immunity following vaccination wanes with time and the odds of pertussis increase by 30% per year after receiving the PSB.

Chapter 7 demonstrates that pertussis is also prevalent among adults with persistent cough and that montelukast may be an effective treatment for persistent cough in patients with pertussis. However, the latter finding is only based on an exploratory subgroup analysis and is therefore not sufficiently robust at this stage to inform the use of montelukast for treating pertussis-induced cough in primary care.

Nevertheless, establishing a diagnosis of pertussis in adults with persistent cough is still of considerable value in terms of providing patients with an explanation for their cough and, if appropriate, starting antibiotic treatment to reduce the duration of the infectious period. The findings reported in chapter 7 suggest that 25% of non-smoking adults who present in primary care with an unexplained or postinfectious persistent cough of two to eight weeks' duration have pertussis. Clinicians should therefore consider confirmatory testing in these patients, particularly if the cough is accompanied by other clinical features of pertussis, such as paroxysmal coughing, inspiratory whooping and post-tussive vomiting(44). Based on surveillance data collected by the London Health Protection Agency between January 2006 and December 2008, 44% of patients aged 15 years and over with clinically suspected pertussis had evidence of recent pertussis infection based on detection of IgG-PT in oral fluid (personal communication, Tim Harrison, London Health Protection Agency, February 2010).

The results of chapter 7 suggest that clinicians should adopt a conservative approach to managing patients who present with acute persistent cough in primary care. Based on the observed changes in Leicester Cough Questionnaire total scores, a clinically relevant degree of spontaneous resolution within two weeks of initial presentation may occur, even without treatment. Clinicians should therefore avoid empirical medication prescribing in

patients with postinfectious persistent cough unless there are other clinical features which suggest additional pathology. Chapter 7 found preliminary data to suggest that montelukast may be an effective treatment for postinfectious cough in adults with atopic conditions, but this finding should also be substantiated further before being adopted as clinical practice.

8.4 Implications of findings for future research

This thesis highlights several important areas for further research to strengthen the evidence base for managing Mp- and pertussis-related postinfectious coughs.

To improve diagnosis and treatment of patients with Mp, large, high quality prospective studies conducted in primary care are needed to develop clinical prediction rules, evaluate the use of rapid diagnostic tests and examine the evidence base for macrolide treatment of confirmed Mp infections.

Clinical prediction rules for diagnosing Mp have previously been published, but involve interpretation of blood results(45) and identification of chest X-ray confirmed pneumonia(46), which may not be possible on initial presentation in most primary care settings. Since primary care clinicians rely mainly on clinical features during the initial consultation, their diagnostic utility should be maximised by clinical prediction rules incorporating data on symptom and sign combinations, character and severity. The diagnostic value of less commonly reported clinical features, such as chest pain and extrapulmonary manifestations, should also be considered.

Given that Mp occurs in cyclical epidemics, clinical prediction rules should incorporate population level data on Mp incidence. The performance of a clinical decision model for pertussis in infants was found to improve with the incorporation of local disease incidence data(47). The influence of concurrent infections on clinical presentation in patients with Mp should also be examined. Optimal detection of multiple infections could be addressed by using laboratory techniques such as 'MassTag' multiplex PCR, which can identify up to 22 respiratory pathogens in a single sample(48).

A novel test based on analysis of throat swabs, which has a diagnostic accuracy of more than 97% compared to PCR and culture, has been proposed as a point of care test for Mp(49). However, the performance of this test has only been evaluated under laboratory conditions. To determine the clinical utility of this test, its feasibility, acceptability and cost-effectiveness should be assessed in primary care, particularly in relation to guiding macrolide prescribing.

However, although macrolides are recommended as first-line treatment for Mp, this is based on the theoretical assumption that beta-lactam antibiotics are unlikely to be effective because the organism has no cell wall(50). There is currently insufficient evidence from trial data to determine the efficacy of macrolides in treating Mp. This is mainly due to the limited availability of published data relating to patients with confirmed Mp infection(51). Trials involving participants with confirmed Mp infections are needed to assess the efficacy of macrolides in treating Mp. These trials should also assess the clinical value and cost-effectiveness of Mp testing before initiating macrolide treatment in patients with acute respiratory tract infections and persistent cough.

Given the current uncertainty which primary care clinicians face in diagnosing and prescribing antibiotics for patients with Mp, further research is needed to examine the relationship between macrolide consumption and incidence of macrolide-resistant Mp in the community. Understanding this relationship will inform public health surveillance of macrolide prescribing and guide antibiotic treatment of Mp, especially during disease outbreaks. Macrolide resistance can be detected based on point mutations in domain V of the 23S rRNA gene(52-54). The incidence of these point mutations should reflect the true incidence of macrolide-resistant strains, since neither plasmids with macrolide resistance encoding erythromycin ribosomal methylase (erm) genes(55) nor enzymes that inactivate macrolides have been found in Mp(56).

Macrolide-resistant Mp has been found during Mp outbreaks in China(57), Italy(58) and Israel(59). During a macrolide-resistant Mp outbreak at a nursery school in China, most Mp-positive children showed no clinical improvement with erythromycin(57). Tetracyclines are clinically effective in treating macrolide-resistant Mp pneumonia, but Mp may remain persistent in the nasopharynx at the end of treatment(60).

Macrolide-resistant Mp may be found in children with persistent cough, for whom GPs frequently consider prescribing antibiotics(61). Macrolide-resistant strains of Mp have been reported in France(62), Italy(58), Japan(63), Germany(64), Israel(59, 65), the United States(66) and China(57, 67, 68). However, attempts to detect evidence of macrolide resistance in the UK have been limited by the number of Mp cases available for testing(69, 70). The London Health Protection Agency is screening Mp cases detected in the cohort described in chapter 6 for evidence of macrolide resistance.

To improve the control of pertussis incidence rates in the community, further research is needed to optimise pertussis vaccination strategy in the UK. Chapter 6 demonstrated that pertussis is still prevalent in school aged children with persistent cough despite high primary and PSB vaccination coverage. To estimate the efficacy and effectiveness of the PSB vaccination and compare different types of PSB vaccines, large, high quality trials with long follow-up periods extending into adolescence are needed. Serological thresholds for immunity after receiving the PSB should also be established to inform optimal timing of an additional booster vaccination.

The need for an additional booster vaccination will also be informed by studies which estimate clinical disease severity in vaccinated pertussis cases. To address this issue, nested pilot work within the cohort discussed in chapter 6 began in July 2012 to measure 24-hour cough frequency using the Leicester Cough Monitor(71) in pertussis-positive children. Cough frequency data have also been obtained from eight adults recruited into the trial described in chapter 7 (four pertussis-positive and four pertussis-negative patients).

An adolescent pertussis booster vaccination was introduced in the United States in 2005, and since then, the ratio of pertussis incidence in adolescents (11 to 18 years old) to that in other age groups has followed a sustained decreasing trend(72). However, further long-term studies are needed to estimate the effect of the adolescent pertussis booster vaccination on disease transmission and healthcare resource utilisation, particularly in relation to infant hospitalisations and deaths. The potential impact on the emergence on new pertussis strains(73) and on the incidence of *Bordetella parapertussis*(74) and other infections associated with a 'pertussis-like' phenotype, should also be studied.

To improve the clinical management of pertussis-induced cough in primary care, more large high quality trials are needed which compare candidate treatments to placebo and other currently prescribed medications(75). Chapter 7 demonstrated that a trial to determine the efficacy of montelukast in patients with pertussis-induced persistent cough would be feasible and worthwhile, particularly over a two-week treatment period.

Although the trial in chapter 7 was conducted in adults, a trial to determine the efficacy of montelukast in children with pertussis should also be considered. Chapter 7 did not recruit children because montelukast is administered to children in the form of chewable tablets or granules, and image-matched placebos were not available for either of these at the time of set-up.

Chapter 6 has shown that pertussis is still prevalent in children with persistent cough. Despite the prolonged time course of pertussis-induced cough, collection of cough duration data using study diaries has been demonstrated to be feasible(19). Quality of life scores and objective cough measures should also be considered. A validated 8-item tool for assessing the effect of children's persistent cough on parental quality of life has recently been developed, with a suggested minimally important difference of 0.9(76). In addition, 4-hour cough frequency measured using the Leicester Cough Monitor correlates highly with 24-hour cough frequency and is responsive to change following trials of therapy(77).

8.5 Summary

The key implications of this thesis for clinical practice are that: 1) diagnosing Mp and pertussis in patients with persistent cough is of considerable value in terms of giving patients an explanation for their cough and informing them about its likely prognosis and 2) clinicians should still consider pertussis in patients who have received their primary and preschool booster vaccinations.

The implications of this thesis for future research are that clinical prediction rules and rapid diagnostic tests are needed to improve the diagnosis of Mp in primary care and a further trial is needed to determine the efficacy of montelukast in the treatment of pertussis-induced cough.

8.6 References

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Appendices

Appendix 1 – Search strategies for systematic review

MEDLINE search strategy

1. Pneumonia/
2. Pneumonia, Bacterial/
3. Pneumonia, Mycoplasma/
4. mycoplasma pneumon*.tw.
5. "m. pneumoniae".tw.
6. (community-acquired pneumon* or community acquired pneumon*).tw.
7. or/1-6
8. Cough/
9. cough*.tw.
10. wheez*.tw.
11. "shortness of breath".tw.
12. sore throat*.tw.
13. coryza.tw.
14. "chest pain".tw.
15. crepitation*.tw.
16. Fever/
17. fever*.tw.
18. Exanthema/
19. (rash or rashes).tw.
20. exp Diarrhea/
21. (diarrhoea or diarrhea).tw.
22. myalgia.tw.
23. Headache/
24. headache*.tw.
25. clinical assessment*.tw.
26. clinical feature*.tw.
27. (symptom* or sign* or characteristic* or manifestation*).tw.
28. or/8-27
29. 7 and 28
30. exp Infant/
31. (infant* or infancy or newborn* or baby* or babies or neonat* or preterm* or prematur*).tw.
32. exp Child/
33. (child* or schoolchild* or school age* or preschool* or kid or kids or toddler*).tw.
34. Adolescent/
35. (adoles* or teen* or boy* or girl*).tw.
36. Minors/
37. Puberty/
38. (minor* or pubert* or pubescen*).tw.
39. exp Pediatrics/
40. (pediatric* or paediatric*).tw.
41. exp Schools/
42. (nursery school* or kindergar* or primary school* or secondary school* or elementary school* or high school* or highschool*).tw.
43. or/30-42

44. 29 and 43
45. Pneumonia, Mycoplasma/di [Diagnosis]
46. Pneumonia, Bacterial/di [Diagnosis]
47. 45 or 46
48. 43 and 47
49. 44 or 48

EMBASE search strategy
1. *PNEUMONIA/
2. bacterial pneumonia/ or infectious pneumonia/
3. Mycoplasma pneumonia/
4. COMMUNITY ACQUIRED PNEUMONIA/
5. mycoplasma pneumon*.tw.
6. “m. pneumoniae”.tw.
7. (community-acquired pneumon* or community acquired pneumon*).tw.
8. 1 or 2 or 3 or 4 or 5 or 6 or 7
9. coughing/ or wheezing/
10. cough*.tw.
11. wheez*.tw.
12. “short of breath*”.tw.
13. “shortness of breath”.tw.
14. sore throat*.tw.
15. coryza.tw.
16. “chest pain”.tw.
17. crepitation*.tw.
18. fever/
19. (fever* or febrile).tw.
20. exp rash/
21. (rash or rashes).tw.
22. diarrhea/
23. (diarrhoea* or diarrhea*).tw.
24. myalgia.tw.
25. HEADACHE/
26. (headache* or head ache*).tw.
27. clinical feature/
28. clinical assessment*.tw.
29. clinical feature*.tw.
30. (symptom* or sign* or characteristic* or manifestation*).tw.
31. 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30
32. 8 and 31
33. exp infant/
34. (infant* or infancy or newborn* or baby* or babies or neonat* or preterm* or prematur*).tw.
35. child/ or boy/ or girl/ or preschool child/ or school child/ or toddler/
36. (child* or schoolchild* or school age* or preschool* or kid or kids or toddler*).tw.
37. adolescent/
38. (adoles* or teen* or boy* or girl*).tw.
39. juvenile/
40. Puberty/

41. (minor* or pubert* or pubescen*).tw.
42. pediatrics/
43. (pediatric* or paediatric*).tw.
44. school/ or high school/ or kindergarten/ or middle school/ or nursery school/ or primary school/
45. (nursery school* or kindergar* or primary school* or secondary school* or elementary school* or high school* or highschool*).tw.
46. 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45
47. 32 and 46

Appendix 2 – Quality assessment tool and coding criteria for systematic review

Item	Yes	No	Unclear
1. Was the spectrum of patients representative of the patients who will receive the test in practice?	Participants recruited prospectively and consecutively from any healthcare setting; diagnosed with CAP; aged 18 years or younger; no serious underlying co-morbidity or immunocompromise*.	Criteria for 'Yes' not met. Participants recruited from limited spectrum of disease severity.	Insufficient information on recruitment method, criteria for diagnosis of CAP and participant characteristics (age, co-morbidity).
2. Is the reference standard likely to classify the target condition correctly?	Positive Mp serology result** +/- use of additional laboratory tests (e.g. culture, PCR).	No use of serology in confirming diagnosis of Mp.	Insufficient information on method of confirming diagnosis of Mp. Discrepant results between serology and other laboratory tests.
3. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Initial serum sample obtained within 24 hours of presentation. Convalescent serum samples obtained 2 to 4 weeks after acute serum samples.	Criteria for 'Yes' not met.	Insufficient information on timing of sample collection.
4. Did the whole sample or a random selection of the sample, receive verification using the intended reference standard? (partial verification avoided)	Attempted to obtain and test serum samples from all study participants. Serology results interpreted as per manufacturers' instructions.	Criteria for 'Yes' not met.	Insufficient information on the number and characteristics of participants from whom serum samples were obtained and tested.
5. Did patients receive the same reference standard irrespective of the index test result? (differential verification avoided)	Same method of laboratory testing for Mp used for all participants.	Choice of laboratory testing for Mp related to clinical symptoms and signs.	Insufficient information on whether choice of method of laboratory testing for Mp was related to participants' clinical symptoms and signs.
6. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)? (incorporation avoided)	Diagnosis of Mp based on laboratory test results only.	Diagnosis of Mp based on laboratory test results and clinical symptoms and signs.	Insufficient information on whether or not diagnosis of Mp was based solely on laboratory test results.

Appendix 2 (continued)

Item	Yes	No	Unclear
7. Were the reference standard results interpreted without knowledge of the results of the index test? (index test results blinded)	Laboratory results interpreted without knowledge of clinical symptoms and signs.	Laboratory results interpreted with knowledge of clinical symptoms and signs.	Insufficient information about whether laboratory results were interpreted with or without knowledge of clinical symptoms and signs.
8. Were the index test results interpreted without knowledge of the results of the reference standard?	Clinical symptoms and signs reported without knowledge of Mp laboratory test results.	Criteria for 'Yes' not met.	Insufficient information about whether clinical features were reported with or without knowledge of Mp laboratory test results.
9. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Data on baseline participant characteristics (age, sex, duration of illness) available but radiological findings not available when data on symptoms and signs were collected.	Criteria for 'Yes' not met.	Insufficient information on whether baseline participant characteristics and/or radiological findings were available when data on symptoms and signs were collected.
10. Were uninterpretable or intermediate test results reported?	Study reported number of participants with intermediate or borderline serology results and/or discrepant results on serology versus other laboratory tests. Study described management of above results during data analysis.	Criteria for 'Yes' not met.	Insufficient information on numbers of participants with intermediate, borderline and discrepant test results and how these were managed during data analysis.
11. Were withdrawals from the study explained?	Number of children who were withdrawn from the study reported together with reasons for withdrawal.	Number of children withdrawn from the study not reported or explained.	Insufficient information about number of children withdrawn from study and reasons for withdrawal.

CAP = community-acquired pneumonia based on clinical +/- radiological criteria; Mp = *Mycoplasma pneumoniae*; PCR = Polymerase Chain Reaction
* *e.g.* cystic fibrosis, bronchiectasis, neoplasia, HIV positive or on immunosuppressant medication.
** Significant rise in antibody titre between paired acute and convalescent sera or high antibody titre on a single serum sample (as per manufacturers' instructions).

8.7 Appendix 3 – publications from thesis

Wang K, Gill P, Perera R, Thomson A, Mant D, Harnden A.
Clinical symptoms and signs for the diagnosis of *Mycoplasma pneumoniae* in children and adolescents with community-acquired pneumonia.
Cochrane Database Syst Rev. 2012;10:CD009175.
doi: 10.1002/14651858.CD009175.pub2.

Wang K, Chalker V, Bermingham A, Harrison T, Mant D, Harnden A.
Mycoplasma pneumoniae and respiratory virus infections in children with persistent cough in England: a retrospective analysis.
Pediatr Infect Dis J. 2011;30(12):1047-51.